Instituto do Cérebro

Universidade Federal do Rio Grande do Norte
Programa de Pós-Graduação em Neurociências

Os efeitos antidepressivos da ayahuasca, suas bases neurais e relação com a experiência psicodélica

Fernanda Palhano Xavier de Fontes

Tese apresentada ao Programa de Pós-Graduação em Neurociências da Universidade Federal do Rio Grande do Norte como requisito parcial para a obtenção do título de Doutor em Neurociências.

Natal-RN, Brasil
Julho de 2017

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Universidade Federal do Rio Grande do Norte - UFRN
Sistema de Bibliotecas - SISBI
Catalogação de Publicação na Fonte. UFRN - Biblioteca Setorial Árvore do Conhecimento - Instituto do Cérebro - ICE

Fontes, Fernanda Palhano Xavier de.
Os efeitos antidepressivos da ayahuasca, suas bases neurais e relação com a experiência psicodélica / Fernanda Palhano Xavier de Fontes. - Natal, 2017.
196f.: il.

Universidade Federal do Rio Grande do Norte, Instituto do Cérebro, Programa de Pós-graduação em Neurociências.
Orientador: Dráulio Barros de Araújo.

1. psicodélicos. 2. ayahuasca. 3. depressão resistente ao tratamento. 4. ensaio clínico randomizado. 5. fMRI. I. Araújo, Dráulio Barros de. II. Título.

RN/UF/Biblioteca Setorial Árvore do Conhecimento - Instituto do Cérebro CDU 616.89-008.454

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Apresentada em 28 de julho de 2017

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Prof. Dr. Dráulio Barros de Araújo – UFRN

Natal-RN
Julho de 2017

Aos voluntários e voluntárias,
anônimos e fundamentais

AGRADECIMENTOS

Aos meus pais e irmãos;
Aos amigos Sérgio, Diego, Vítor, Lara, Suzana, Lízie, Clarissa,
às meninas da Escola e aos colegas do ICe;
Ao NeuroImago;
Às companheiras do LabGirls: Heloísa, Jéssica, Kátia e Morgana;
Aos fóruns do SPM e FSL, ao Sci-Hub;
A todos que colaboraram com este projeto: como voluntários, nas coletas e discussões;
Ao Hospital Universitário Onofre Lopes e seus funcionários;
Em especial, a Dráulio, orientador e grande parceiro de aventuras nesses mais de cinco anos de DMAOi.

RESUMO

Ensaios clínicos recentes têm sugerido o potencial antidepressivo de alguns psicodélicos serotonérgicos, como a ayahuasca e a psilocibina. A ayahuasca é uma substância utilizada há centenas de anos por povos indígenas da bacia amazônica. Essa bebida é geralmente preparada a partir de duas plantas, uma contendo a triptamina psicodélica N,N-dimetiltriptamina, um agonista de receptores serotonérgicos e sigma-1. A outra planta contém potentes inibidores reversíveis da monoamina oxidase A, como a harmina, harmalina e tetrahidroharmina. Em um ensaio clínico recente, 17 pacientes com depressão resistente ao tratamento foram submetidos a uma única sessão com a ayahuasca, e a severidade dos sintomas avaliados por escalas clínicas para a depressão, nesse caso a Escala de Avaliação de Depressão de Montgomery-Åsberg (MADRS) e a escala de Hamilton (HAM-D). Os resultados sugerem melhora significativa já nas primeiras horas após a ingestão, efeito esse mantido por 21 dias. Apesar de promissor, esse estudo não controlou o efeito placebo, que é alto, chegando a alcançar 40%, em ensaios clínicos para depressão. Esta tese faz parte de um ensaio clínico duplo-cego randomizado placebo-controlado em 35 pacientes com depressão unipolar resistente ao tratamento. Um grupo de voluntários controles saudáveis também foi avaliado. Os pacientes receberam uma única dose de ayahuasca ou placebo. Um dia antes e um dia depois da administração da substância, os pacientes participaram de uma série de testes e avaliações, incluindo escalas psicométricas e imagem por ressonância magnética funcional (fMRI), realizados no decurso de uma sessão experimental de quatro dias. A severidade da depressão foi aferida por duas escalas clínicas: MADRS e HAM-D. As avaliações foram realizadas um dia antes (linha de base), e um, dois e sete dias após a sessão de tratamento. Observamos efeitos antidepressivos significativos da ayahuasca quando comparados ao placebo em todos os instantes de tempo avaliados (D1, D2 e D7). Os escores da MADRS foram significativamente menores no grupo da ayahuasca, em comparação com o placebo. Da mesma forma, as pontuações da HAM-D foram significativamente diferentes entre os grupos no D7. Os tamanhos do efeito entre os grupos aumentaram de D1 para D7. As taxas de resposta (redução de 50% nos sintomas) foram elevadas para ambos os grupos nos dias D1 e D2. Já sete dias após a sessão, a taxa de resposta foi significativamente maior no grupo ayahuasca que no placebo (64% ayahuasca x 27% placebo). Nesse mesmo ponto de avaliação, a taxa de remissão foi marginalmente maior no grupo ayahuasca que no placebo ($p<0.056$, 36% ayahuasca x 7% placebo). Além disso, investigamos a relação entre os efeitos antidepressivos e os efeitos psicodélicos agudos da ayahuasca, avaliados pela Escala de Avaliação Alucinógena (HRS, *Hallucinogenic Rating Scale*) e o Questionário de Experiência Mística (MEQ30, *Mystical Experience Questionnaire*). Os resultados sugerem que efeitos psicodélicos mais intensos estão correlacionados a taxas de resposta maiores no D7. Por fim, para investigar as bases neurais dos efeitos antidepressivos da ayahuasca, utilizamos dados de fMRI adquiridos durante a realização de um protocolo de regulação emocional um dia antes e um dia após a sessão de tratamento. Observamos mudanças na amígdala, ínsula anterior, córtex pré-frontal ventrolateral e na porção subgenual do giro do cíngulo. Este é o primeiro ensaio clínico, duplo-cego, controlado, a testar o uso de uma substância psicodélica para a depressão resistente ao tratamento. Em conjunto, nossos resultados trazem novas evidências que apoiam a segurança e o valor terapêutico da ayahuasca, administrada dentro de um cenário apropriado, para ajudar a tratar a depressão.

ABSTRACT

Recent open label trials show that psychedelics hold promise as fast-onset antidepressants in treatment resistant depression. In Brazil, a psychedelic substance worth mentioning is the ayahuasca. This brew composed by the psychedelic N,N-dimethyltryptamine (N,N-DMT), a serotonin and sigma-1 receptors agonist, and reversible monoamine oxidase A inhibitors, such as harmine, harmaline, and tetrahydroharmine, causes changes in perception, emotion and cognition. Preliminary results from our group suggest its antidepressant potential, in addition to demonstrating its safety and tolerability. Although promising, none of these studies have controlled for the placebo effect, which is high in clinical trials for depression. To address this issue, we conducted a parallel-arm, double-blind randomized placebo-controlled trial in 35 patients with treatment-resistant depression. A healthy control group of 50 volunteers was also examined. Patients received a single dose of either ayahuasca or placebo. One day before dosing, and one day after dosing patients were enrolled in a series of tests and assessments, including psychometric scales and fMRI, conducted in the course of a four-day experimental session, to assess changes in different markers of depression. Two clinical scales for depression assessed changes in depression severity: the Montgomery–Åsberg Depression Rating Scale (MADRS) and the Hamilton Depression Rating scale (HAM-D). Assessments were made at baseline, and at 1, 2 and 7 days after dosing. We observed significant antidepressant effects of ayahuasca when compared to placebo at all endpoints. MADRS scores were significantly lower in the ayahuasca group, compared to placebo. Likewise, HAM-D scores were significantly different at D7. Between-group effect sizes increased from D1 to D7. Response rates were high for both groups at D1 and D2, and were significantly higher in the ayahuasca group at D7. Remission rate was marginally significant at D7. In addition, we examined the relationship between the antidepressant and acute psychedelic effects of ayahuasca. The psychedelic effects were assessed using the Hallucinogen Rating Scale (HRS) and the Mystical Experience Questionnaire (MEQ30) after the dosing session. We found that higher psychedelic effects measured by both HRS and MEQ30 correlated with improvements in clinical outcome at D7. Finally, to investigate the neural bases of the antidepressant effects of ayahuasca, we used fMRI data through an emotional task at baseline, and one day after dosing. We observed changes in key limbic regions, such as amygdala, anterior insula, ventrolateral prefrontal cortex, and subgenual cingulate cortex in ayahuasca group when compared to placebo. To our knowledge this is the first controlled trial to test a psychedelic substance in treatment resistant depression. Overall, this study brings new evidence supporting the safety and therapeutic value of ayahuasca, dosed within an appropriate setting, to help treat depression.

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LISTA DE ABREVIAÇÕES

ACC – *Anterior cingulate cortex*
ART – *Artifact detection tools*
CONEP – Comissão nacional de ética em pesquisa
DMN – *Default mode network*
DSM – *Diagnostic and Statistical Manual of Mental Disorders*
EEG – Eletroencefalografia
EPI – *Echo-planar imaging*
EQ – *Experience Questionnaire*
FD – *Frame displacement*
FFMQ - *Five Facets Mindfulness Questionnaire*
fMRI – Imagem funcional por ressonância magnética
HAM-D – *Hamilton depression rating scale*
HPA – *Hypothalamic–pituitary–adrenal*
HUOL – Hospital universitário Onofre Lopes
IAPS – *International Affective Picture System*
LORETA – *Low Resolution Brain Electromagnetic Tomography*
MADRS – Montgomery–Åsberg Depression Rating Scale
MAOi – Inibidores de monoamina oxidase
Pc - *Precuneus*
PCC – *Posterior cingulate cortex*
RCT – *Randomized clinical trials*
SCID – *Structured Clinical Interview for DSM*
*S*PECT – *Single-photon emission computed tomography*
SSRI – *Selective serotonin reuptake inhibitor*
TCA – Antidepressivos tricíclicos
UDV – União do vegetal

Considerações Iniciais

A pesquisa com psicodélicos ganhou renovado fôlego nos últimos anos, com diversos centros espalhados pelo mundo investigando como essas substâncias interagem com o cérebro, alterando aspectos cognitivos, afetivos e perceptuais da mente humana, bem como seu potencial no tratamento de transtornos mentais (Tupper et al. 2015, Sessa 2015).

No Brasil, uma substância psicodélica que merece destaque é a ayahuasca. Utilizada tradicionalmente por povos indígenas da região amazônica, ela foi introduzida nos centros urbanos por meio de religiões sincréticas, como a Barquinha, o Santo Daime e a União do Vegetal (UDV) (Tupper and Labate 2014). Em 1985, essa bebida conquistou caráter legal de uso ritualístico (Labate and MacRae 2016). Atualmente, o uso da ayahuasca se expandiu, sendo possível encontrar igrejas em diferentes partes do mundo (Labate and Jungaberle 2011).

A forma mais frequente de preparo da ayahuasca se faz pela decocção de duas plantas amazônicas: *Banisteriopsis caapi* e *Psicotria viridis*. Do ponto de vista farmacológico, a ayahuasca se assemelha a outros psicodélicos clássicos. A *P. viridis* é rica em N,N-dimetiltriptamina (N,N-DMT), uma triptamina alucinógena que atua principalmente como agonista serotonérgico, particularmente do receptor 5HT-2A (Carbonaro and Gatch 2016), além de atuar sobre receptores sigma-1 (Fontanilla et al. 2009). A N,N-DMT quando administrada por via oral é degradada por enzimas monoamina oxidase (MAO) presentes no trato gastrintestinal. A *B. caapi*, no entanto, contém beta-carbolinas, particularmente a harmalina e a harmina, que são inibidores reversíveis da MAO-A (iMAO-A). Além disso, a bebida possui tetrahidroharmina (THH) um fraco inibidor da recaptação de serotonina (McKenna, Callaway, and Grob 1998). A presença dessas substâncias faz com que a N,N-DMT não seja completamente degradada, permitindo que chegue à corrente sanguínea, ultrapasse a barreira hematoencefálica, e leve a efeitos no sistema nervoso central (Callaway et al. 1999). Além disso, as beta-carbolinas aumentam a disponibilidade de outras monoaminas no cérebro, contribuindo para os efeitos da ayahuasca (Schenberg et al. 2015).

Os efeitos provocados pela bebida duram aproximadamente 4 horas, e incluem alterações somáticas, como mudanças na percepção da temperatura corporal e sensações de formigamento na pele, leveza do corpo, inquietação motora e bocejos (Shanon 2002a, Riba, Rodriguez-Fornells, et al. 2001). Fenômenos visuais também são bastante frequentes e ocor-

rem principalmente com os olhos fechados, na forma de imagens mentais, que vão desde padrões sem conteúdo semântico (e.g. figuras caleidoscópicas, com a presença ou não de cores) até cenas complexas, vívidas como em um sonho (Shanon 2002b, de Araujo et al. 2012). Além de distorções sensoriais, ampla gama de efeitos são frequentemente descritos e incluem mudanças no processo de pensamento, exacerbação emocional (às vezes com choro e/ou risadas), alterações de humor, estado de maior ciência *(awareness*), mudanças no fluxo de pensamentos, alteração da percepção de tempo, da percepção de si próprio (*self*), e do senso de realidade (Callaway et al. 1999, Riba, Rodriguez-Fornells, et al. 2001, Shanon 2002a).

Desde 2006 nosso grupo tem atuado em três frentes de pesquisa envolvendo a ayahuasca: as bases neurais dos efeitos agudos, subagudos, e seu potencial antidepressivo. Em uma primeira série de experimentos, utilizamos a imagem funcional por ressonância magnética (fMRI, do inglês, *Functional Magnetic Resonance Imaging*) para avaliar o impacto da ayahuasca nos sistemas neurais associados à geração de imagens mentais visuais (*visual mental imagery*) (de Araujo et al. 2012), e à rede de modo padrão (DMN, do inglês, *Default Mode Network*) (Palhano-Fontes et al. 2015). Encontramos mudanças em sistemas associados à memória, como o hipocampo, à visão, incluindo o córtex visual primário, e ligados à ciência do estímulo (*awareness*), como as regiões frontopolares, BA10 (de Araujo et al. 2012). Identificamos, ainda, redução da atividade na maior parte da DMN, bem como redução de conectividade funcional no giro posterior do cíngulo/precuneus (PCC/Pc) (Palhano-Fontes et al. 2015).

Em colaboração com o grupo do prof. Jordi Riba, do Instituto Sant Pau, Barcelona/Espanha, utilizamos a imagem por ressonância magnética para avaliar mudanças estruturais no cérebro associadas ao grau de experiência com a ayahuasca. Encontramos aumento da espessura cortical no córtex pré-frontal medial (mPFC, *medial Prefrontal Cortex*), e redução da espessura no PCC/Pc. Além disso, foi encontrada correlação significativa entre a redução da espessura cortical no PCC/Pc com o traço de personalidade conhecido como abertura (*openness*) (Bouso et al. 2015).

Em colaboração com pesquisadores da Faculdade de Medicina de Ribeirão Preto/USP, em dois trabalhos coordenados pelo prof. Jaime Hallak buscamos avaliar o potencial antidepressivo da ayahuasca (Osório et al. 2015, Sanches et al. 2016). Em um primeiro ensaio

aberto (*open label trial*), seis pacientes com depressão refratária participaram de uma única sessão de tratamento com ayahuasca. Os pacientes foram avaliados por escalas clínicas tradicionalmente utilizadas para medir a severidade dos sintomas de depressão, a Escala de Avaliação de Depressão de Montgomery-Åsberg (MADRS, *Montgomery-Åsberg Depression Ratting Scale*) e a escala de Hamilton (HAM-D), antes, durante e após os efeitos agudos da ayahuasca. Foi observada redução significativa dos sintomas 24h após a sessão, que se mantiveram reduzidos por 21 dias (Osório et al. 2015). Na continuidade desse ensaio clínico, o número de pacientes foi ampliado para 17, as escalas clínicas para depressão foram aplicadas, e mudanças subagudas de fluxo sanguíneo cerebral foram avaliadas por SPECT (*Single Photon Emission Computed Tomography*) 8h após uma única sessão de tratamento com ayahuasca. Novamente, foi observada redução significativa dos sintomas de depressão já durante a sessão experimental (40 min pós ingestão), que permaneceu significativa até 21 dias após a sessão. Além disso, foi observado aumento de fluxo sanguíneo no núcleo accumbens, ínsula direita e área subgenual do cíngulo anterior (Sanches et al. 2016).

Embora promissores, esses dois estudos possuem uma limitação importante: ambos utilizaram desenho aberto, que não controla o efeito placebo, que chega a beneficiar até 40% dos pacientes em ensaios clínicos para depressão quando submetidos a tratamento com substância placebo (Sonawalla and Rosenbaum 2002).

Motivados por essa limitação, desde meados de 2010 iniciamos o planejamento de um ensaio randomizado duplo-cego placebo-controlado, e em janeiro de 2014 iniciamos a fase de coleta de dados, após aprovação do projeto pelo comitê de ética em pesquisa do Hospital Universitário Onofre Lopes (HUOL – UFRN, parecer n° 579.479).

Neste projeto tivemos dois grupos experimentais: 50 voluntários saudáveis (grupo controle) e 35 pacientes com depressão. Metade dos voluntários receberam ayahuasca, metade placebo. Em fevereiro de 2016 concluímos a aquisição de dados do grupo controle, e em junho de 2016 concluímos a aquisição de dados dos pacientes.

Os efeitos antidepressivos foram avaliados por duas escalas clínicas (HAM-D e MADRS), antes, logo após e, mês a mês, durante seis meses após a sessão de tratamento. Além do efeito antidepressivo, este projeto foi desenhado para avaliar outros efeitos, agudos e subagudos da ayahuasca. Durante a sessão de tratamento (efeitos agudos) os voluntários foram

avaliados por eletroencefalografia (EEG), escalas psiquiátricas, questionários, e medidas de cortisol salivar. Para avaliar os efeitos subagudos, realizamos as seguintes medidas um dia antes e um dia após as sessões de tratamento: escalas psiquiátricas, testes neuropsicológicos, avaliação do discurso, imagem por ressonância magnética (estrutural e fMRI), eletroencefalografia durante o sono e medidas bioquímicas, incluindo cortisol, BDNF (*Brain Derived Neurotrophic Factor*), fator de necrose tumoral alfa (TNF-a, *Tumor Necrosis Factor alpha*), e as interleucinas IL-4, IL-6, e IL-10.

Esta tese está inserida nesse projeto maior e tem como objetivo principal avaliar os efeitos antidepressivos da ayahuasca em pacientes com depressão resistente ao tratamento. Além disso, pretende-se buscar por relações entre o efeito antidepressivos da ayahuasca, a experiência psicodélica, avaliada por escalas, e mudanças subagudas de fMRI. As demais avaliações realizadas estão sendo analisadas em trabalhos separados, alguns já em andamento.

A parte escrita deste trabalho está dividida em cinco seções principais. A primeira delas é uma breve introdução, baseada em um capítulo de livro que publicamos durante esta tese (apêndice A), que apresenta parte do racional para se explorar os efeitos antidepressivos da ayahuasca, bem como os efeitos agudos, subagudos e os achados preliminares dos efeitos antidepressivos da ayahuasca. Em seguida, são apresentados os objetivos da tese. Apesar da tese não conter resultados de todos os dados coletados, resolvemos deixar neste documento o registro do protocolo experimental inteiro, de modo que a seção de material e métodos é mais abrangente que o protocolo desta tese em si. A seção de resultados é composta por três artigos que contemplam nossos achados. No primeiro artigo são apresentados os resultados do ensaio clínico randomizado duplo-cego placebo-controlado, que avaliou os efeitos antidepressivos da ayahuasca por escalas clínicas para depressão (HAM-D e MADRS). O segundo artigo investiga a relação entre os efeitos antidepressivos da ayahuasca, e a experiência psicodélica aguda. No terceiro trabalho, avaliamos mudanças neurais medidas por fMRI, e como essas mudanças se correlacionam com os efeitos antidepressivos observados. Por último, fazemos uma discussão geral dos nossos resultados, retomando os resultados individuais de cada artigo, e trazendo perspectivas de continuação do projeto.

Introdução

2.1. Depressão maior: causas e diagnóstico

A depressão maior (MDD, do inglês *Major Depressive Disorder*) é um transtorno de alta prevalência, sendo duas vezes mais frequente em mulheres que em homens e que usualmente inicia-se na terceira década de vida. Para a maioria das pessoas, apresenta-se em episódios recorrentes, embora cerca de 20-25% tenham a doença cronicamente (Fava and Kendler 2000). Está associada a intenso sofrimento pessoal, a altas taxas de morbidade e aumento da mortalidade (Ebmeier, Donaghey, and Steele 2006). Um estudo da Organização Mundial de Saúde aponta a depressão como a quarta maior causa de morbidade e prevê que em 2020 ela será a segunda doença com maior impacto sobre a vida produtiva (Fava and Kendler 2000).

De acordo com o Manual de Diagnóstico e Estatística dos Transtornos Mentais da Associação Americana de Psiquiatria (DSM–IV-TR), o diagnóstico de episódio depressivo exige a presença de humor deprimido e/ou anedonia por período mínimo de duas semanas acompanhado por pelo menos quatro dos seguintes sintomas: perda ou ganho de peso considerável (5% do peso); agitação ou retardo psicomotor; insônia ou hipersonia; fadiga ou diminuição de energia; baixa autoestima ou culpa inapropriada; dificuldade de pensar, de concentração ou de tomar decisões e pensamentos de morte; ideação ou tentativas de suicídio. Para o diagnóstico é necessário, ainda, que esses sintomas estejam causando sofrimento significativo e/ou prejuízo social, ocupacional ou de outras áreas de funcionamento, e não devem ser diretamente causados por outra condição médica geral, uso de substância psicoativa, nem preencherem critério para episódio misto, em que os critérios diagnósticos de depressão e mania são preenchidos concomitantemente (American Psychiatric Association. 2000).

Existem algumas teorias que procuram explicar a etiologia da MDD, e até bem pouco tempo a mais aceita era a teoria monoaminérgica, segundo a qual a depressão teria causa na diminuição de monoaminas, particularmente a serotonina (Wong and Licinio 2001). Embora a teoria monoaminérgica seja a mais consolidada, ela não responde a algumas questões importantes como, as causas do distúrbio monoaminérgico e a refratariedade ao tratamento antidepressivo com fármacos que disponibilizam monoaminas na fenda sináptica. Assim, diversas outras hipóteses têm surgido, como, por exemplo, a de que a MDD tem suas raízes no sistema imunológico (Li, Soczynska, and Kennedy 2011), ou a da hipercortisolemia, que suge-

re que a hiperatividade crônica do eixo Hipotálamo-Hipófise-Adrenal (HPA, do inglês, *Hypothalamic-Pituitary-Adrenal*) pode levar à depressão (Kunugi et al. 2012). De fato, essas três hipóteses parecem estar intimamente ligadas uma vez que o aumento de citocinas pró-inflamatórias podem modificar o funcionamento do eixo HPA e também diminuir a disponibilidade de triptofano, que por sua vez levaria à redução de monoaminas (Cai, Huang, and Hao 2015, Maes 2011).

Além da dificuldade em identificar sua etiologia, outro desafio importante em relação ao diagnóstico da MDD é a ausência de um biomarcador específico relacionado a essa doença. O uso de biomarcadores tem um papel importante na compreensão e monitoramento de tratamentos convencionais e alternativos (Leuchter et al. 2010). O consenso atual é que nenhum biomarcador sozinho é suficiente para prever e identificar indivíduos em risco, fazer o diagnóstico ou tomar decisões clínicas diretas sobre a MDD. Por conseguinte, recomenda-se a construção de um painel multimodal de biomarcadores para obter a precisão necessária na prática clínica ou nos protocolos de pesquisa (Lakhan, Vieira, and Hamlat 2010). Atualmente, existem vários candidatos para a identificação de biomarcadores para a MDD, incluindo avaliação neuropsicológica, EEG, EEG durante o sono, marcadores bioquímicos, imagens médicas, genômica, proteômica e metabolômica, dentre outros. A seguir apresentaremos alguns desses possíveis biomarcadores e como eles estão alterados na MDD.

2.1.1. Marcadores bioquímicos

A informação sobre marcadores bioquímicos é acessada indiretamente por medições do líquido cefalorraquidiano, saliva ou sangue. Nesse cenário, o líquido cefalorraquidiano pode ser considerada a fonte que melhor reflete a atividade cerebral. No entanto, não é facilmente acessível sem risco. Por outro lado, embora a urina e as fezes sejam facilmente coletadas, é difícil associar os níveis de metabólitos presentes nesses dois veículos com os encontrados no cérebro. A saliva também pode ser usada com sucesso para a medição de hormônios esteroides, como o cortisol (Dziurkowska, Wesolowski, and Dziurkowski 2013). Marcadores bioquímicos também foram buscados no sangue, tanto por sua fácil acessibilidade quanto pelo fato de que muitas moléculas encontradas no cérebro são excretadas através da via de circulação sanguínea.

Um dos achados mais frequentes é a alta concentração de cortisol no plasma sanguíneo de pacientes com MDD, de acordo com a teoria da hipercortisolemia (Leonard 2007, Tadić et al. 2011). Além disso, alterações nos níveis plasmáticos de citocinas, como por exemplo redução da concentração de citocinas anti-inflamatórias (IL-4 e IL-10) e aumento da concentração de citocinas pró-inflamatórias (IL-1, IL-6 e TNF-α), são observadas em pacientes com MDD, e também são consideradas biomarcadores confiáveis, de acordo com a teoria inflamatória (Li, Soczynska, and Kennedy 2011).

2.1.2. Marcadores de EEG durante o sono

Anormalidades nos padrões de sono são constantemente reveladas nos achados de EEG em pacientes que sofrem de MDD. Embora não haja um único marcador de sono especificamente associado à MDD, muitos estudos encontraram diferenças no sono entre pacientes com MDD e indivíduos saudáveis. O uso do EEG durante o sono em pacientes com MDD revelou diminuição da continuidade do sono, latência (intervalo entre o início do sono e a ocorrência do primeiro período) reduzida para o sono REM (do inglês, *Rapid Eye Movement*), aumento da quantidade de sono REM, maior período de sono REM, aumento da densidade de sono REM (número de movimentos oculares durante o sono REM) e diminuição do sono de ondas lentas (Benca et al. 1992, Riemann, Berger, and Voderholzer 2001, Tsuno, Besset, and Ritchie 2005).

A redução da latência do sono REM associada à MDD tem sido observada desde a década de 1970. Vários estudos relatam que essa mudança está relacionada à secreção de cortisol durante a noite, sugerindo que a desregulação do humor, do sono e do eixo HPA podem estar interligadas (Poland et al. 1992). Uma relação inversa é observada entre o nível de cortisol e o encurtamento de latência: os indivíduos que apresentam a latência para o sono REM mais curta também têm o maior grau de atividade do eixo HPA (Asnis et al. 1983). Especula-se que a associação entre a latência do REM e o eixo HPA é causada por uma desregulação do sistema colinérgico muscarínico, que exerce um papel em ambos os sistemas fisiológicos (Asnis et al. 1983).

Além disso, foi proposto que a diminuição da latência para o sono REM associada à MDD pode ser um traço familiar (Giles, Roffwarg, and Rush 1987). Em uma investigação sobre fatores de risco em pacientes com histórico de depressão foi observado que o risco rela-

tivo de MDD em parentes com latência do REM reduzida era quase três vezes maior do que para parentes com latência do REM não reduzida (Giles et al. 1988). Também foi observada latência do REM reduzida, mesmo em parentes do primeiro grau psicologicamente assintomáticos de pacientes deprimidos (Giles et al. 1989). Alguns estudos ainda relataram distúrbios do sono (Lauer et al. 1995) e desregulação do eixo HPA (Mannie, Harmer, and Cowen 2007) em adultos saudáveis, mas cujos parentes de primeiro grau tinham histórico de MDD.

Além disso, a conexão entre o sono REM e a MDD foi demonstrada pelo fato de que a privação aguda do sono (sono total, parcial ou especificamente REM) alivia os sintomas depressivos (Benca et al. 1992, Riemann, Berger, and Voderholzer 2001). Sabe-se também que o tratamento farmacológico influencia o sono REM. Por exemplo, os antidepressivos diminuem a quantidade de sono REM e mudanças nos estágios iniciais do tratamento antidepressivo pode ser um preditor do resultado do tratamento (Riemann, Berger, and Voderholzer 2001).

2.1.3. Marcadores de neuroimagem

O uso de imagens por ressonância magnética tem ajudado a identificar os mecanismos psicopatológicos subjacentes à MDD. Estudos recentes mostram que pacientes deprimidos apresentam alterações neuroanatômicas e funcionais quando comparados a controles saudáveis. Um dos resultados mais consistentes é a redução do volume do hipocampo em pacientes com MDD (Bremner et al. 2000, Lorenzetti et al. 2009, MacQueen 2009). Outro achado comum é a redução do volume em algumas das estruturas dos núcleos da base, como o caudado e o putâmen (Lorenzetti et al. 2009). Embora os resultados não sejam sempre convergentes, tais mudanças morfológicas são uma indicação de que as imagens estruturais podem fornecer informações relevantes para a caracterização da MDD.

Além das alterações da anatomia cerebral, alguns estudos utilizaram técnicas de neuroimagem funcional para avaliar a função cerebral de pacientes com MDD. A amígdala, uma estrutura do lóbulo temporal medial que é bastante sensível a estímulos emocionais, tem sido repetidamente implicada em sintomas de MDD. Estudos usando fMRI mostraram hiperatividade da amígdala em pacientes deprimidos (Davey et al. 2011, Sheline et al. 2001). A porção subgenual do córtex cingulado anterior (sgACC) também parece estar envolvida na MDD. De forma consistente, estudos encontram aumento da atividade metabólica dessa área em paci-

entes com MDD quando comparados aos controles saudáveis (Mayberg et al. 1997, Drevets, Savitz, and Trimble 2008).

Além disso, o córtex pré-frontal ventromedial, o córtex cingulado anterior e o córtex parietal inferior têm sido objeto de muitos estudos sobre MDD. Essas regiões constituem uma rede conhecida como DMN, caracterizada por maior atividade durante o repouso do que durante a execução de uma tarefa com objetivo específico (Raichle et al. 2001). A DMN tem sido envolvida em processos que envolvem autojulgamento, recordação de memórias autobiográficas, simulações mentais, distúrbios mentais e *daydreaming* (Buckner, Andrews-Hanna, and Schacter 2008, Northoff et al. 2006). Usando uma tarefa de regulação emocional, Sheline e colegas examinaram a funcionalidade da DMN em pacientes com MDD (Sheline et al. 2009). Os resultados mostraram que os indivíduos com depressão não conseguiram modular diferentes regiões da DMN, incluindo o córtex cingulado anterior, o córtex parietal lateral, o córtex pré-frontal medial e o córtex temporal lateral (Sheline et al. 2009). Além disso, alguns estudos mostraram alterações nos padrões de conectividade funcional da DMN. Por exemplo, Greicius et al. (2007) relataram um aumento significativo na conectividade funcional no córtex cingulado subgenual e precuneus em pacientes deprimidos quando comparados com controles saudáveis (Greicius et al. 2007). Esses resultados são consistentes com os sintomas clínicos, como a ruminação, em que os pacientes apresentam pensamentos negativos recorrentes (Devilbiss, Jenison, and Berridge 2012).

Diferenças na conectividade funcional de outras redes também foram encontradas em pacientes depressivos (Anand et al. 2005, Sheline et al. 2010, Veer et al. 2010). Por exemplo, Veer e colegas mostraram que existe: (i) uma diminuição da conectividade entre a amígdala e a insula anterior esquerda, estruturas relacionadas a estímulos emocionais; (ii) redução na conectividade do pólo frontal esquerdo, uma rede associada à atenção e memória de trabalho; E (iii) uma diminuição da conectividade bilateral do giro lingual e de áreas visuais (Veer et al. 2010). Alterações na conectividade também têm sido relacionadas à resposta ao tratamento antidepressivo. Por exemplo, um estudo avaliou o efeito da sertralina, um antidepressivo comumente utilizado, na conectividade córtico-límbica (Anand et al. 2005). Após o tratamento, os pacientes apresentaram maior conectividade entre o córtex cingulado anterior e regiões límbicas, um circuito associado à regulação emocional (Anand et al. 2005).

2.2. Tratamentos da depressão maior

Várias alternativas estão disponíveis para o tratamento da MDD. Dentre elas, cabe destacar o uso de medicações antidepressivas (Uppal et al. 2010), psicoterapia (Cuijpers et al. 2011) e a eletroconvulsoterapia (Andrade et al. 2010). O objetivo do tratamento, em especial do farmacológico, é a remissão do episódio depressivo, definida pelo DSM-IV como a ausência de sinais e sintomas significativos por pelo menos dois meses ou pontuação em escalas de severidade de depressão como a Hamilton (HAM-D $\leq$ 7) (Hamilton 1960) e Montgomery–Åsberg Depression Rating Scale (MADRS $\leq$ 10) (Montgomery and Asberg 1979, Kennedy et al. 2001).

Em geral o tratamento medicamentoso passa por diferentes fases. A primeira, conhecida como aguda, dura cerca de 8 semanas e é determinante para avaliar a resposta do paciente ao tratamento (Bolland and Keller 2004). Se essa primeira etapa for bem sucedida, há um período de continuação que dura de 16 a 20 semanas e tem como objetivo prevenir possíveis recaídas do episódio depressivo (Kennedy et al. 2001). Alguns casos passam pela fase de manutenção por tempo indeterminado, indicada a indivíduos com alto risco de recorrência, como aqueles que já apresentaram episódios ou resposta parcial ao tratamento e pacientes com episódios de elevada gravidade, com sintomas psicóticos e risco suicida (American Psychiatric Association. 2000).

Várias classes de antidepressivos estão disponíveis no mercado, e são classificados de acordo com sua estrutura química, seu efeito na sinapse e sua ação sobre o sistema de recaptação e metabolização dos neurotransmissores (Ebmeier, Donaghey, and Steele 2006).

As primeiras medicações da classe de antidepressivos foram descobertas casualmente durante o desenvolvimento de antihistamínicos (imipramina) e tuberculostáticos (iproniazida) (Guimaraes and Graeff 2000). O reconhecimento de que a imipramina bloqueava a recaptação de noradrenalina (NA) e de serotonina (5HT), somado à observação que a anfetamina, que libera noradrenalina e diminui sua recaptação neuronal, tem efeito euforizante, levaram à proposição, na década de 60, de que a depressão seria causada pela diminuição de noradrenalina e de serotonina (Wong and Licinio 2001).

Os antidepressivos de primeira geração são divididos em tricíclicos (TCA) e inibidores da monoamina oxidase (MAOi). Ambos agem aumentando o nível extracelular de monoami-

nas, os tricíclicos pela inibição da recaptação de dopamina, serotonina e noradrenalina, e os MAOi pela inibição do metabolismo desses neurotransmissores (Bolland and Keller 2004). Além da ação terapêutica, os TCA atuam em vários outros receptores, apresentando efeitos antimuscarínicos, antihistamínicos e anti-α2 adrenérgicos que levam a resultados indesejáveis como retenção urinária, constipação intestinal, hipotensão ortostática, ganho de peso e sonolência. Além disso, os TCA bloqueiam canais de sódio interferindo na condução nervosa sendo potencialmente arritmogênicos. Em relação aos MAOi, o principal efeito colateral é o risco de crises hipertensivas desencadeadas pela sua ingestão combinada à de alimentos contendo tiramina, uma amina simpatomimética presente em grande quantidade em certos alimentos, que é metabolizada pela MAO. Desse modo, pacientes em uso de MAOi devem fazer dieta pobre em tiramina, evitando alimentos como queijos, bebidas alcoólicas, alimentos curados, envelhecidos, em conserva, frios e alguns vegetais, como banana e abacate (Guimaraes and Graeff 2000).

Dada a baixa seletividade dos antidepressivos de primeira geração, novos antidepressivos foram desenvolvidos, Dentre estes estão os inibidores seletivos da recaptação de serotonina – (SSRI, *Selective Serotonin Reuptake Inhibitors*) (fluoxetina, paroxetina, dentre outros), inibidores seletivos da recaptação de noradrenalina (reboxetina), inibidores da recaptação de serotonina e noradrenalina (venlafaxina, desvenlafaxina e duloxetina). Existem ainda antidepressivos com múltiplos mecanismos de ação, como a mirtazapina, que age como antagonista noradrenérgico α2 pré-sináptico e antagonista de receptores de serotonina (5HT2 e 5HT3), e a nefazodona que age tanto inibindo a recaptação de serotonina e noradrenalina, como antagonizando receptores α2 e 5HT (Cipriani et al. 2009).

Esses novos antidepressivos, possuem essencialmente o mesmo mecanismo de ação das primeiras medicações e atuam aumentando o nível de monoaminas na fenda sináptica (Cai, Huang, and Hao 2015). Essa nova geração de antidepressivos é mais segura, e os efeitos colaterais, embora menores, ainda estão presentes e são específicos para as diferentes classes das medicação. Por exemplo, a disfunção sexual e distúrbios gastrintestinais são comuns pelo uso dos SSRI, e sonolência e ganho de peso estão associados ao uso da mirtazapina (Cipriani et al. 2009). Tendo em vista que a eficácia entre os diversos antidepressivos disponíveis é semelhante, a escolha do medicamento é feita ponderando-se os efeitos colaterais, segurança, tole-

rabilidade, preferências individuais do paciente, qualidade de ensaios clínicos disponíveis e o custo (American Psychiatric Association. 2000).

Apesar do progresso substancial no desenvolvimento de novos medicamentos, menos de 50% dos pacientes alcançam remissão após um único tratamento antidepressivo, e mesmo após quatro tratamentos sistematicamente aplicados, cerca de um terço não consegue alcançar remissão (Warden et al. 2007). Os antidepressivos atuais ainda carregam outra limitação: a demora de várias semanas para atingir os efeitos terapêuticos desejados (Cai, Huang, and Hao 2015).

Tendo essas questões como pano de fundo, enorme esforço tem sido dedicado na busca por novos antidepressivos, visando melhorar a eficiência do tratamento, acelerar o início dos efeitos terapêuticos, e reduzir efeitos colaterais (Uppal et al. 2010). Estudos recentes com a cetamina, um anestésico antagonista dos receptores N-metil-d-aspartato (NMDA) do sistema glutamatérgico, mostraram efeito antidepressivo após injeção intravenosa única, que persistiu significantemente após uma semana (Liebrenz et al. 2007). Além disso, ensaios randomizados utilizando cetamina em pacientes com depressão resistente ao tratamento mostraram que a substância leva a uma redução nos sintomas depressão que é máxima um dia após a administração, e que permanece significativa por até sete dias (Romeo et al. 2015).

Outro exemplo é o uso da (+/−)-1-(2,5-Dimethoxy-4-iodophenyl)-2-aminopropane (DOI), um potente agonista 5HT2A/2C. Em um estudo, camundongos submetidos ao teste do nado forçado, paradigma utilizado para estudar depressão em roedores, exibiram diminuição do tempo de imobilidade 6 horas após receberem DOI, efeito que é considerado preditivo de resposta antidepressiva (Masuda and Sugiyama 2000).

Outro agonista serotonérgico, a N, N-dimetiltriptamina (DMT), também tem sido considerado potencial antidepressivo. Algumas hipóteses têm surgido de que, em doses baixas, o mecanismo ansiolítico ligado à DMT seria mediado pelo receptor de amina-traço, que poderia ser um dos sítios de ação da DMT endógena (Jacob and Presti 2005).

Efeitos ansiolíticos e antidepressivos estão também relacionados às beta-carbolinas. Por exemplo, o uso de harmina em modelo de depressão em roedores leva à redução de vários sinais e sintomas associados a quadros depressivos, como anedonia, e regulação para níveis de normalidade do hormônio adrenocorticotrofina e de BDNF hipocampal, cuja expressão e re-

gulação têm sido associada a fisiopatologia da depressão (Fortunato et al. 2010a, Fortunato et al. 2010b, Cai, Huang, and Hao 2015).

Com base nas evidências apresentadas aqui, e em dois estudos realizados por nosso grupo, descritos abaixo, surgiu a questão do uso de substâncias que combinam DMT e iMAO, como é o caso da ayahuasca, no tratamento da MDD. Existem vários relatos anedóticos de que o uso ritual da ayahuasca está associado ao alívio dos sintomas da depressão.

2.3. Ayahuasca

Tradicionalmente a ayahuasca é preparada pela decocção da casca e tronco da *Banisteriopsis caapi* com folhas da *Psychotria viridis*. A *B. caapi* contém os alcaloides harmina, tetrahidroharmina (THH) e, em menor quantidade, a harmalina, os três pertencentes ao grupo das β-carbolinas. Por outro lado, a *P. viridis* fornece a triptamina alucinógena DMT (Riba et al. 2003, Mckenna, Towers, and Abbott 1984).

Os efeitos da ayahuasca dependem da interação sinérgica entre esses componentes. As β-carbolinas são potentes iMAOs reversíveis (Mckenna, Towers, and Abbott 1984) e podem aumentar os níveis de serotonina, bloqueando sua deaminação. Sua principal ação na ayahuasca é proteger a DMT da degradação periférica, prevenindo a deaminação oxidativa da DMT ingerida oralmente e permitindo que ela atinja o sistema nervoso central (Riba et al. 2003, Mckenna, Towers, and Abbott 1984). Os efeitos farmacológicos da DMT também dependem da sua interação com o sistema serotoninérgico. A DMT é o substrato para transportadores de absorção de serotonina de superfície celular (SERTs) e transportadores de monoamina de vesículas neuronais (VMAT2). Ao contrário dos fármacos que são inibidores da absorção, a DMT é transportada para o citosol ou vesícula por SERT ou VMAT2, respectivamente (Cozzi et al. 2009). Portanto, altas concentrações intracelulares e vesiculares de DMT podem ser alcançadas dentro dos neurônios e podem interagir com os receptores sigma-1 intracelulares localizados na membrana reticular endoplasmática associada à mitocôndria (Su, Hayashi, and Vaupel 2009). Assim, a DMT pode ser liberada na fenda sináptica após fusão vesicular para interagir com receptores sigma-1 de superfície celular ou receptores pós-sinápticos de serotonina (Cozzi et al. 2009).

Os efeitos da ayahuasca são heterogêneos e abrangem mudanças sensoriais, cognitivas e afetivas (Riba, Rodriguez-Fornells, et al. 2001), experiências visuais ricas (Shanon 2002b,

Shanon 2002a) e experiências enteógenas (Shanon 2003). Esses efeitos começam entre 35 e 40 minutos após a ingestão de chá, atingindo o pico entre 90 e 120 min, e com duração de aproximadamente 4 h (Riba, Rodriguez-Fornells, et al. 2001). Até o momento, todos os estudos até o momento demonstraram a segurança da ayahuasca, com relatos de indivíduos que o usaram há mais de 30 anos sem evidência de danos à saúde (Callaway et al. 1999, Grob et al. 1996, Riba, Rodriguez-Fornells, et al. 2001, Riba et al. 2003) e não provoca alterações significativas na pressão arterial - sistólica, diastólica e média e frequência cardíaca (Riba et al. 2003). Além disso, mostrou-se que o uso ritual do chá não está associado aos problemas psicossociais que geralmente são encontrados com outras drogas (Fábregas et al. 2010).

2.3.1. Efeitos agudos da ayahuasca

A maior parte dos estudos realizados com a ayahuasca investigou seus efeitos agudos, quase todos em indivíduos com experiência prévia com ayahuasca e/ou outros psicodélicos. Em recente revisão sistemática da literatura, foram encontrados 28 trabalhos publicados, até dezembro de 2016, que avaliaram mudanças provocada pela ingestão de ayahuasca por humanos, desse total, 18 são sobre efeitos agudos (Dos Santos et al. 2016).

A caracterização desses efeitos agudos muito se dá pela aplicação de escalas psicométricas (questionários) sensíveis a certas características do estado psicodélico, como, por exemplo, às mudanças de percepção visual. A *Hallucinogenic Rating Scale* (HRS) é uma delas (Grob et al. 1996, Callaway et al. 1999, Riba, Rodríguez-Fornells, et al. 2001, Riba, Rodriguez-Fornells, et al. 2001, McKenna 2005, Alonso et al. 2015). Ela foi inicialmente proposta para caracterizar os efeitos causados pela administração intravenosa de N,N-DMT em voluntários experientes (Strassman et al. 1994). Ela é dividida em seis sub-escalas[1], efeitos somáticos, afeto, volição, cognição, percepção e intensidade, e tem sido utilizada em diversos estudos com diferentes psicodélicos (Riba et al. 2003, Strassman et al. 1994, Ross et al. 2016, Griffiths et al. 2016).

O primeiro estudo a utilizar a HRS como instrumento de avaliação dos efeitos agudos da ayahuasca foi feito com membros experientes da UDV, uma das igrejas que utiliza a ayahuasca como sacramento (Grob et al. 1996). Os resultados mostram aumento significativo em todas as sub-escalas da HRS (Grob et al. 1996). Esses resultados são consistentes com outros

[1]Essa divisão foi baseada no conteúdo das perguntas, e não em análise fatorial exploratória.

estudos controlados com voluntários experientes, e que também encontraram aumento significativo nas seis sub-escalas da HRS durante os efeitos agudos da ayahuasca liofilizada (Alonso et al. 2015, Riba, Rodriguez-Fornells, and Barbanoj 2002, Riba, Rodríguez-Fornells, et al. 2001). Os efeitos são dependentes da dose de N,N-DMT contida na formulação. Em cinco das seis sub-escalas da HRS (à exceção de volição), quanto maior a dose, maiores os efeitos detectados pela HRS (Riba, Rodriguez-Fornells, et al. 2001, Riba, Rodriguez-Fornells, and Barbanoj 2002, 2003). Por outro lado, a administração de doses consecutivas de ayahuasca, cada uma contendo 0.75 mg de N,N-DMT/kg, sugere ausência de tolerância e de sensibilização[2] (Dos Santos et al. 2012).

Testes neuropsicológicos também têm sido usados para avaliar os efeitos agudos da ayahuasca. Um desses estudos comparou a performance de usuários ocasionais e experientes, sob efeito de uma única dose de ayahuasca, em três testes neuropsicológicos: *Stroop*, *Sternberg* e Torre de Londres (Bouso et al. 2013). Nos dois grupos foram observados aumento do número de erros na tarefa de *Sternberg*, diminuição do tempo de reação e manutenção da acurácia na tarefa *Stroop*. Já no teste Torre de Londres houve aumento significativo nos tempos de execução, de resolução, e no número de movimentos realizados pelos voluntários inexperientes, mas não nos voluntários experientes. Além disso, foi observada correlação significativa inversa entre a piora de desempenho na Torre de Londres e tempo de uso de ayahuasca, ou seja, quanto mais experiente menor a degradação da resposta (Bouso et al. 2013).

Já a identificação de correlatos neurais dos efeitos agudos da ayahuasca tem sido realizada, principalmente, por diferentes técnicas de neuroimagem funcional, como a imagem funcional por ressonância magnética (fMRI) (de Araujo et al. 2012, Palhano-Fontes et al. 2015), SPECT (Riba et al. 2006) e eletroencefalografia (EEG) (Don et al. 1998, Alonso et al. 2015, Hoffmann, Hesselink, and Barbosa 2001, Riba et al. 2002, Riba et al. 2004, Stuckey, Lawson, and Luna 2005, Schenberg et al. 2015).

A avaliação por SPECT durante os efeitos da ayahuasca em indivíduos experientes sugere aumento significativo de fluxo sanguíneo bilateralmente na ínsula anterior, no cíngulo anterior/córtex frontomedial à direita, na amígdala e giro parahipocampal esquerdos (Riba et al. 2006).

[2]Tolerância é a necessidade de doses cada vez maiores para se obter o mesmo efeito de antes. Sensibilização é o processo em que alguns efeitos da substância se manifestam com doses cada vez menores.

Por outro lado, a avaliação das visões (*mirações*) por fMRI em voluntários experientes revelou mudanças significativas em sistemas associados à percepção visual, incluindo o córtex visual primário, à memória, como o hipocampo, e regiões associadas à ciência (*awareness*) e à saliência de estímulos, como áreas frontopolares (BA10) (de Araujo et al. 2012). Nesse mesmo grupo de voluntários foi possível identificar redução da atividade na maior parte da DMN, bem como redução de conectividade funcional no giro posterior do cíngulo/precuneus (PCC/Pc) (Palhano-Fontes et al. 2015).

Nos estudos que utilizaram EEG, o correlato mais consistentemente encontrado durante o efeito agudo da ayahuasca tem sido a redução da potência na banda de frequência alfa (Don et al. 1998, Riba et al. 2002, Riba et al. 2004, Schenberg et al. 2015). Estudos que comparam o registro de EEG de voluntários antes e após a ingestão de ayahuasca liofilizada encontraram redução significativa das potências nas bandas alfa, beta, delta e teta (Riba et al. 2002, Riba et al. 2004). Utilizando a técnica de LORETA (*low-resolution electromagnetic tomography*) foi possível observar que as alterações em alfa, beta e delta eram localizadas predominantemente em regiões em volta da junção temporoparietooccipital, enquanto as alterações em teta se deram no córtex temporomedial e regiões frontomediais (Riba et al. 2004) (riba 2004). Recentemente, um estudo observou além da redução da potência de alfa 50 minutos após a ingestão de ayahuasca, aumento da potência de gama, sugerindo um efeito bifásico induzido pela bebida (Schenberg et al. 2015).

2.3.2. Efeitos subagudos da ayahuasca

Os efeitos subagudos da ayahuasca, e os de longo prazo, têm sido pouco estudados. Efeitos subagudos são geralmente avaliados no intervalo entre 24h e 48h após ingestão da bebida.

Um desses estudos investigou se o uso repetido da ayahuasca – 4 a 5 vezes distribuídas ao longo de 2 semanas – levaria a mudanças de criatividade visual. Quarenta indivíduos experientes completaram testes de criatividade entre antes da primeira sessão (linha de base) e entre 24-48h após o final da última sessão. Comparado a um grupo que não participou das sessões (N=21), a ingestão repetida de ayahuasca no contexto ritual aumentou significativamente o número de soluções originais, o que sugere aumento de criatividade visual (Frecska et al. 2012).

Outro estudo avaliou mudanças subagudas em traços de *mindfulness*[3] após uma sessão com ayahuasca (Soler et al. 2016). Os resultados sugerem mudanças tanto no questionário de cinco facetas *mindfulness* (FFMQ, *Five Facets Mindfulness Questionnaire*) quanto no questionário de experiências (EQ, *Experience Questionnaire*), que vão no mesmo sentido das mudanças observadas após práticas meditativas (Soler et al. 2014). Foram observados aumentos significativos em duas facetas da FFMQ relacionadas à redução no julgamento das experiências, redução de reatividade interior, e aumento significativo da capacidade de descentramento (*decentering*) medida pela EQ (Soler et al. 2016).

Em estudo recente, essas mudanças de traços de *mindfulness* foram associadas a alterações neurometabólicas e de conectividade funcional durante a fase subaguda dos efeitos da ayahuasca (Sampedro et al. 2017). Dados de espectroscopia por ressonância magnética sugerem alteração subaguda de glutamato/glutamina, creatina e N-acetilaspartato/N-acetilaspartilglutamato, todos no córtex cingulado posterior (PCC). Além disso, foi observada correlação entre a redução de glutamato/glutamina e a redução previamente observada na sub-escala "non-judgment" do FFMQ. Os dados de *resting state* revelaram aumento significativo da conectividade funcional entre o PCC e o córtex cingulado anterior (ACC), e entre o ACC e estruturas límbicas à direita, que por sua vez se apresentou correlacionada ao aumento de escores no questionário de autocompaixão (Sampedro et al. 2017).

2.3.3. Ayahuasca e os biomarcadores de depressão maior

Além das evidências farmacológicas descritas anteriormente, outras evidências também podem apoiar o uso da ayahuasca como antidepressivo. Estudos recentes indicaram que o uso regular de ayahuasca está envolvido na modulação de longo prazo dos sistemas de serotonina no cérebro, especificamente nos níveis de SERTs (Cozzi et al. 2009). Essa modulação pode aumentar a função do sistema serotoninérgico, o que pode ser um mecanismo para seu possível efeito positivo em pacientes com MDD. A ayahuasca também tem efeitos significativos no sistema endócrino, principalmente no eixo HPA, e no sistema imunológico. Estudos têm encontrado aumentos nos níveis de prolactina e cortisol aproximadamente 2 horas após uma única dose de ayahuasca (Dos Santos et al. 2011). Esse aumento do cortisol tem impacto

[3]O construto *mindfulness* é associado ao estado mental ligado à habilidade de manter ciência (*awareness*) no presente, reconhecendo e aceitando seus pensamentos, sentimento e sensações corporais.

na imunidade celular e reduz os linfócitos CD3 e CD4 após o uso da ayahuasca (Dos Santos et al. 2011). Outro estudo usou duas doses sequenciais de ayahuasca com intervalo de 4 horas e encontrou efeitos endócrinos e imunomoduladores análogos aos relatados anteriormente (Dos Santos et al. 2012). De acordo com essa evidência, o aumento rápido dos níveis de cortisol foi observado em um único tratamento agudo com antidepressivos como a reboxetina que atua no sistema serotoninérgico (Schüle 2006).

O efeito da ayahuasca no sono também foi avaliado em voluntários saudáveis após uma administração única da dose de ayahuasca durante o dia (Barbanoj et al. 2008). Os resultados indicaram que a ayahuasca não teve efeitos significativos na iniciação ou continuidade do sono, conforme avaliado por medidas subjetivas e objetivas. Além disso, verificou-se que a bebida inibe o sono REM, diminuindo sua duração em valores absolutos e em porcentagem do sono REM. Uma tendência no aumento na latência REM também foi relatada (Barbanoj et al. 2008). Com base nesses resultados, pode-se especular ainda que a ayahuasca pode ter potencial terapêutico na MDD atuando em mecanismos de sono, já que a literatura aponta que as alterações de sono na depressão (aumento da quantidade de sono REM e redução da latência REM) vão na direção oposta às mudanças induzidas pela ayahuasca (Benca et al. 1992, Riemann, Berger, and Voderholzer 2001, Tsuno, Besset, and Ritchie 2005).

Além disso, há também evidências de fMRI sobre os potenciais efeitos antidepressivos da ayahuasca. Em um estudo realizado por nosso grupo, que objetivou avaliar as mudanças induzidas pela ayahuasca na DMN, indivíduos saudáveis foram avaliados por fMRI antes e durante os efeitos da ayahuasca. Observou-se que a ayahuasca causou uma redução no sinal fMRI dos nós centrais da DMN, como o córtex cingulado anterior, o córtex pré-frontal medial, o córtex cingulado posterior, o precuneus e o lobo parietal inferior (Palhano-Fontes et al. 2015). Além disso, observaram-se mudanças nos padrões de conectividade da DMN, especialmente uma diminuição na conectividade funcional do precuneus (Palhano-Fontes et al. 2015). Esses achados são contrários aos de alguns estudos que mostram uma modulação oposta em pacientes com depressão, isto é, aumento da atividade e conectividade funcional da DMN (Greicius et al. 2007, Sheline et al. 2009).

Recentemente, ensaios clínicos do tipo aberto (*open label trials*) foram conduzidos a fim de avaliar o potencial antidepressivo da ayahuasca. Em uma primeira avaliação, seis pa-

cientes com depressão refratária participaram de uma única sessão de tratamento com ayahuasca. Os pacientes foram avaliados por escalas clínicas, tradicionalmente utilizadas para medir a severidade dos sintomas depressivos (HAM-D e MADRS), antes, durante e após os efeitos agudos da ayahuasca. Foi observada redução significativa dos sintomas 24h após a sessão, mantendo-se reduzidos por 21 dias (Osório et al. 2015). Em seguida, o número de pacientes foi ampliado para 17 e mudanças subagudas de fluxo sanguíneo cerebral foram avaliadas por SPECT, 8h após a experiência com ayahuasca (Sanches et al. 2016). Novamente, foi observada redução significativa dos sintomas de depressão, dessa vez, já durante a sessão experimental (40 min pós ingestão), permanecendo significativa por 21 dias. Além disso, foi observado aumento de fluxo sanguíneo no núcleo accumbens, ínsula direita e área subgenual do cíngulo anterior, todas envolvidas na fisiopatologia da depressão (Sanches et al. 2016, Otte et al. 2016).

Embora os resultados desses ensaios sejam promissores, todos possuem uma limitação importante: a utilização de desenho aberto que não controla o efeito placebo. Em ensaios clínicos para depressão, esse efeito pode beneficiar até 40% dos pacientes (Sonawalla and Rosenbaum 2002). Dessa forma, este trabalho propõe a avaliar o potencial antidepressivo da ayahuasca utilizando um desenho randomizado, duplo-cego, placebo-controlado em pacientes com depressão resistente ao tratamento. Além disso, procuramos entender como os efeitos antidepressivos se relacionam com os efeitos psicodélicos agudos e alterações neurais causados pela ayahuasca.

Objetivos

3.1. Objetivos gerais

Avaliar e explicar os efeitos antidepressivos da ayahuasca em pacientes com depressão resistente ao tratamento.

3.2. Objetivos específicos

- Avaliar o potencial terapêutico da ayahuasca em pacientes com depressão resistente ao tratamento utilizando um ensaio clínico randomizado, duplo-cego, placebo-controlado por meio de escalas psiquiátricas (HAM-D e MADRS);
- Investigar relações entre os efeitos antidepressivos da ayahuasca, pelas escalas HAM-D e MADRS, e os efeitos psicodélicos agudos, avaliados pelas escalas HRS e MEQ30;
- Avaliar alterações subagudas da ayahuasca utilizando dados de fMRI em regiões cerebrais relacionadas ao processamento emocional em pacientes com depressão resistente ao tratamento;
- Investigar relações entre mudanças observadas nos dados de fMRI, durante o protocolo de regulação emocional e mudanças nos sintomas de depressão.

Material & Métodos

4.1. Aspectos éticos

Este projeto foi realizado de acordo com a Declaração de Helsinque (1964) e a resolução CNS 466/12. Ele foi aprovado pelo comitê de ética em pesquisa do Hospital Universitário Onofre Lopes (HUOL – UFRN) e pela Comissão Nacional de Ética em Pesquisa (CONEP), parecer n° 579.479.

4.2. Indivíduos controle e pacientes com depressão

Pacientes. 35 pacientes com presença de episódio depressivo diagnosticado por meio de entrevista clínica estruturada para o DSM-IV eixo I (SCID), caracterizado como tendo depressão resistente ao tratamento[4], com idade entre 18 e 60 anos. Os seguintes critérios de exclusão foram adotados: **(i)** experiência prévia com ayahuasca, **(ii)** diagnóstico de doença clínica atual, baseado em anamnese, exame físico e laboratorial, **(iii)** gravidez, **(iv)** histórico de doenças neurológicas, **(v)** histórico de transtorno afetivo bipolar ou esquizofrenia, **(vi)** histórico de mania ou hipomania, **(vii)** uso de substâncias de abuso, **(viii)** risco suicida iminente, **(ix)** apresentar redução de 50% ou mais nos escores da HAM-D e/ou MADRS entre o *washout* e o primeiro dia de coleta, **(x)** apresentar redução de 50% ou mais nos escores da MADRS, entre a avaliação de linha de base (*baseline*) e o dia seguinte, da sessão de tratamento.

Controles. 50 voluntários sem diagnóstico de depressão com idade entre 18 e 60 anos, pareados ao grupo de pacientes com respeito aos critérios sócio-demográficos. Os seguintes critérios de exclusão foram adotados para o grupo controle: **(i)** experiência prévia com ayahuasca, **(ii)** diagnóstico de doença clínica atual, baseado em anamnese, exame físico e laboratorial, **(iii)** gravidez, **(iv)** histórico de doenças neurológicas/psiquiátrica, **(v)** uso de substâncias de abuso, **(vi)** impossibilidade de realizar exame de ressonância magnética.

4.3. As substâncias: ayahuasca e placebo

A ayahuasca foi administrada em forma líquida, fornecida gratuitamente por uma filial da igreja da Barquinha, sediada em Ji-Paraná-RO. A substância utilizada como placebo foi idealizada e produzida em conjunto com o Laboratório de Farmacognosia, da Faculdade de Farmácia (UFRN), de maneira a reproduzir algumas propriedades organolépticas da aya-

[4]definida como persistência dos sintomas depressivos após intervenção com pelo menos dois antidepressivos comerciais de diferentes classes.

huasca como sabor amargo e azedo, e cor marrom. Para cada 50 ml de água foram adicionados 5 mg de levedo de cerveja, 1 mg de ácido cítrico, 1 mg de sulfato de zinco, e aproximadamente 40 gotas de corante caramelo. A presença de sulfato de zinco confere propriedades eméticas ao placebo.

Cada voluntário recebeu 1 ml/kg de placebo ou ayahuasca, ajustadas de modo a conter 0.36 mg/Kg de N,N-DMT na dose recebida. As concentrações de N,N-DMT, harmina, harmalina e THH foram determinadas por cromatografia pelo laboratório de análises toxicológicas da Universidade de São Paulo (USP), coordenado pelo prof. Maurício Yonamine. As primeiras dosagens, realizadas em abril de 2016, revelaram os seguintes valores para duas amostras distintas da ayahuasca do estudo:

	N-N-DMT	**Harmina**	**Harmalina**	**THH**
amostra 1	0,37 mg/mL	1,97 mg/mL	0,21 mg/mL	1,15 mg/mL
amostra 2	0,35 mg/mL	1,75 mg/mL	0,26 mg/mL	1,24 mg/mL

4.4. Escalas clínicas, questionários e exames

4.4.1. Avaliação de sintomas de depressão e mania

Avaliamos os sintomas de depressão por meio das seguintes duas escalas clínicas, aplicadas por uma equipe de quatro psiquiatras, e que foram utilizadas para diagnóstico e quantificação da severidade de depressão:

1. *Entrevista clínica estruturada para o DSM–IV eixo I* (SCID-I), versão paciente – instrumento utilizado para diagnóstico de doenças psiquiátricas (DelBen, Rodrigues, and Zuardi 1996), e na triagem dos voluntários controle e pacientes;
2. *Escala de avaliação para depressão de Hamilton* (HAM-D, *Hamilton Depression Rating Scale*) – avalia severidade de sintomas depressivos no intervalo de uma semana (Hamilton 1960). Utilizamos a versão com 21 itens que avaliam humor, sentimentos de culpa, ideação suicida, insônia, agitação ou retardo, ansiedade, perda de peso e sintomas somáticos. Apenas os escores dos 17 primeiros itens são somados para indicar a severidade dos sintomas, de acordo com os seguintes estratos: ausência de sintomas depressivos (entre 0-7), depressão leve (8-13), depressão moderada (14-18), depressão severa (19-22) e depressão muito severa (≥23).

3. *Escala de depressão de Montgomery-Åsberg* (MADRS, *Montgomery–Åsberg depression rating scale*) – avalia severidade de sintomas depressivos em 10 itens. Foi criada em 1979 com o objetivo de ser mais sensível aos efeitos provocados por diferentes tratamentos e o resultado diz respeito ao instante da sua aplicação (Montgomery and Asberg 1979). O somatório dos escores pode variar entre 0-60, indicando ausência de sintomas depressivos (entre 0-10), depressão leve (11-19), depressão moderada 20-34 e depressão severa (≥35).
4. Escala de Avaliação de Mania de Young (YMRS-MV, do inglês *Young Mania Rating Scale*) – é utilizada para avaliação da gravidade de sintomas maníacos. Foi utilizada versão traduzida e adaptada para o português (Vilela et al. 2005).

4.4.2. Questionários sensíveis à experiência psicodélica

Exploramos os efeitos agudos da ayahuasca por meio dos seguintes questionários, dois deles desenhados para avaliação de experiências psicodélicas, um que explora características da estrutura do pensamento, bem como três escalas clínicas sensíveis a sintomas psicóticos, de mania, e de estados dissociativos:

1. *Hallucinogenic Ratting Scale* (HRS) – essa escala foi desenvolvida para avaliar os efeitos subjetivos induzidos pela N,N-DMT (Strassman et al. 1994). Ela é dividida em 6 fatores: intensidade percepção, afeto, cognição, somestesia e volição. Utilizamos uma versão com 100 questões traduzidas e adaptadas para o português (Mizumoto et al. 2011).
2. *Mystical Experience Questionnaire* (MEQ) – essa escala foi proposta por Pahnke (1969) como ferramenta de investigação sensível aos efeitos agudos de psicodélicos, particularmente associados a experiências místicas. A versão revisada (MEQ30) manteve 30 itens e contempla 4 fatores (Maclean et al. 2012): (i) místico (unidade, qualidade noética e caráter sagrado), (ii) humor positivo, (iii) transcendência do espaço/tempo, (iv) inefabilidade.
3. *Amsterdam Resting-State Questionnaire* (ARSQ) – é composta por 54 itens que avaliam aspectos cognitivos do estado de repouso. Ela engloba dez dimensões: descontinuidade da mente, teoria da mente, si-próprio (*self*), planejamento, sonolência, confor-

to, interocepção, preocupação com a saúde, pensamento verbal, pensamento visual (Diaz et al. 2014).

4. Escala clínica de avaliação de estados dissociativos (CADSS, do inglês *Clinician-Administered Dissociative States Scale*) – avalia manifestações de desrealização e dissociação (Bremner et al. 1998). Esses sintomas incluem lapsos de memória (amnésia), experiências fora do corpo ou outras distorções corporais (despersonalização), distorções visuais, como visão em túnel (desrealização) e fragmentação do sentido de si-próprio (distúrbio de identidade). É composta por 27 itens dos quais 19 são objetivos e oito são pontuados por observação feita pelo médico psiquiatra, e está baseada em critérios mais subjetivos.
5. Escala Breve de Avaliação Psiquiátrica (BPRS, do inglês *Brief Psychiatric Rating Scale*): essa escala pode ser utilizada para avaliar a presença de sintomas psiquiátricos como depressão, ansiedade, alucinação e comportamento não usual. É composta por 18 itens pontuados de 1-7. É dividida em quatro dimensões: sintomas positivos, sintomas negativos, ansiedade/depressão e ativação (sintomas do tipo maníaco) (Zuardi et al. 1994).

4.5. Desenho experimental

Este projeto foi desenhado como um ensaio randomizado, duplo-cego, placebo-controlado (http://clinicaltrials.gov/NCT02914769). Metade dos voluntários bebeu ayahuasca, e a outra metade, placebo. A ordem do tratamento foi definida de maneira aleatória, utilizando-se a técnica de permutação em blocos (www.randomization.com). Os sujeitos de cada grupo (controle e paciente) foram numerados por ordem de participação e randomizados por blocos de 10 indivíduos.

Os voluntários foram recrutados a partir de propaganda boca a boca entre psiquiatras, clínicas e consultórios de atendimento psiquiátrico, e pela divulgação do projeto na mídia e redes sociais. Para seleção dos voluntários e acompanhamento dos pacientes, foi criado um ambulatório semanal no serviço de psiquiatria do Hospital Universitário Onofre Lopes (HUOL). Os interessados foram primeiramente triados por telefone/e-mail. Uma vez pré-selecionados, os voluntários foram encaminhados ao ambulatório de psiquiatria para triagem final pela equipe de psiquiatria e verificação dos critérios de inclusão/exclusão.

Os pacientes selecionados passaram por um período sem uso de medicação antidepressiva (**Figura 1**). Esse procedimento, conhecido por *washout*, é comum durante a troca de medicação, a fim de evitar interação medicamentosa. O período de *washout* depende do tempo de meia vida da medicação em uso (Grunebaum et al. 2003). Por exemplo, para a fluoxetina, um inibidor seletivo da receptação de serotonina, esse período é de 2 semanas. Somente ao final do período de *washout* é que os pacientes participaram do experimento, e na semana seguinte, iniciaram novo esquema de tratamento.

O projeto foi desenhado de modo a permitir avaliar efeitos agudos, subagudos e antidepressivos da ayahuasca. Os efeitos antidepressivos foram acessados pela HAM-D e MADRS em vários instantes (descritos adiante) antes, durante e ao longo de seis meses, após a sessão de tratamento. Durante os efeitos agudos da ayahuasca os voluntários foram monitorados por eletroencefalografia (EEG), escalas psiquiátricas, questionários, e coleta de saliva, para avaliação de cortisol. Os efeitos subagudos da ayahuasca foram acessados por um conjunto de medidas realizadas 24h antes e 24h após as sessões de tratamento. Essas medidas incluíram EEG durante o sono, imagem por ressonância magnética (estrutural e fMRI), escalas psiquiátricas, testes neuropsicológicos, questionários e medidas bioquímicas (cortisol, BDNF, TNF-α, IL-4, IL-6, IL-10).

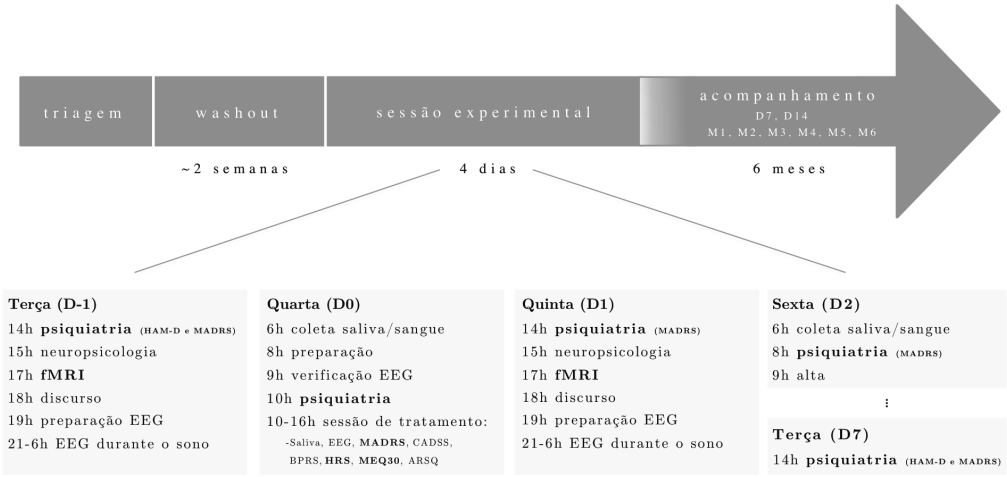

Figura 1. **Cronograma da coleta de dados para cada voluntário.** A primeira etapa consistia em passar por uma triagem. Apenas os pacientes passaram por um período de *washout* (retirada da medicação). A etapa de coleta de dados tinha duração de quatro dias. Após essa fase, os pacientes continuaram sendo acompanhados por seis meses.

A **Figura 1** mostra o cronograma da coleta de dados para cada um dos voluntários. Um dia antes da sessão de tratamento (D-1), sempre às terças-feiras por volta das 14h, os

voluntários foram recebidos por um membro da equipe, quando deveriam ler os termos de consentimento livre e esclarecido (TCLE), de autorização para gravação de voz e imagem, e assiná-los, caso estivessem de acordo. Após preenchimento do questionário epidemiológico, eles foram submetidos, sequencialmente e ao longo da tarde, às avaliações de linha de base, que incluíam, exame clínico e aplicação de escalas psiquiátricas (HAM-D e MADRS), avaliação neuropsicológicas, imagem funcional por ressonância magnética (fMRI, *functional Magnetic Resonance Imaging*), e registro do discurso.

As avaliações neuropsicológicas foram selecionadas e aplicadas por equipe coordenada pelo prof. João Carlos Alchieri, do Departamento de Psicologia da UFRN. Nas duas avaliações (antes e após tratamento) foram aplicados três testes. O **teste do relógio**, para avaliar alguns aspectos de funções cognitivas, motoras, e executivas (Colombo et al. 2009), **o teste de trilhas**, para explorar aspectos de atenção sustentada, o rastreamento visual, destreza motora e memória operacional (da Mota 2008), e o **teste de dígitos**, que se propõe a avaliar atenção e memória de trabalho (Wechsler 2004).

O protocolo de imagem por ressonância magnética será descrito em mais detalhes adiante. Em resumo, nas duas sessões (antes e após tratamento) de MRI (*Magnetic Resonance Imaging*) os indivíduos realizaram dois protocolos experimentais: **(i)** uma tarefa de regulação emocional e **(ii)** um protocolo de *resting-state*.

A coleta dos discursos ocorreu logo após as sessões de MRI (antes e após tratamento). Os registros foram realizados por gravador do tipo MP3, para serem em seguida transcritos pela empresa *Audiotext* (Curitiba – PR). Em cada uma das sessões fizemos cinco registros de discurso. Quatro deles se referiam a histórias que deveriam ser criadas a partir de imagens afetivas extraídas do banco de dados IAPS (*International Affective Picture System*) (Lang, Bradley, and Cuthbert 2008). Os indivíduos criavam e contavam duas histórias de imagens negativas, e duas de imagens neutras. O quinto relato se referia à descrição dos pensamentos que recordavam ter tido durante o protocolo de *resting state*.

Após o registro dos discursos, já era noite e os indivíduos seguiam para o jantar. Em seguida, dava-se início à montagem e preparação do sistema de EEG. Os registros foram realizados em um ambiente do laboratório de neuroimagem funcional (NeuroImago), projetado para ser um quarto de dormir confortável. Utilizamos um sistema de 32 canais (Brain Pro-

ducts, EasyCap, Herrsching-Breitbrunn, Alemanha), com frequência de amostragem 1kHz e o eletrodo FCz como referência. Durante toda noite foi feito registro contínuo de eletroencefalograma (EEG, 25 canais), eletroculograma (EOG, 2 canais), eletrocardiograma (ECG, 2 canais), e eletromiograma (EMG, 3 canais). Os voluntários foram acompanhados durante toda noite por dois profissionais, pelo menos um deles com formação em enfermagem. Além desse registro, as escalas de sonolência de Epworth (Johns 1991), e o índice de qualidade de sono de Pittsburg (Buysse et al. 1989) foram aplicados para avaliar características de sono dos voluntários.

Às 6h da manhã do dia seguinte (4ª feira), despertávamos os voluntários para realizar coleta de saliva e sangue, em jejum. As coletas de saliva foram feitas pelo próprio voluntário por meio de material específico (*Salivette®*, da Sarstedt, Alemanha). Coletamos, ainda, 30 ml de sangue que serviram para avaliar **(i)** os seguintes dados clínicos de rotina: hemograma completo, glicemia de jejum, triglicerídeos, LDL, HDL e colesterol total, sódio e potássio plasmáticos, ureia e creatinina, proteína c-reativa, TGO, TGP e beta-HCG para mulheres em idade fértil, e **(ii)** enviado para o laboratório de Medidas Hormonais do departamento de fisiologia da UFRN, coordenado pela Profa. Nicole Galvão-Coelho, para dosagem de citocinas pró-inflamatórias incluindo interleucinas-4 (IL-4), IL-6 e IL-10, de cortisol plasmático, de fator de necrose tumoral alfa (TNF-α), de fator neurotrófico derivado do cérebro (BDNF).

Após leve café da manhã[5], os voluntários eram preparados para a sessão de tratamento, durante a qual receberiam ayahuasca ou placebo. Cada sessão durava aproximadamente 8 horas, indo geralmente das 8h às 16h. Durante esse período, os voluntários foram monitorados por eletroencefalografia (EEG), acessados por escalas psiquiátricas (MADRS, BPRS, CADSS, YMRS), e feitas coletas de saliva, para medida de cortisol. Ao final da sessão de tratamento, os voluntários preencheram a HRS, foram entrevistados, quando deveriam contar livremente a experiência, e em seguida, avaliados pela equipe de psiquiatria e liberados quando estivessem se sentindo confortáveis.

Na tarde do dia seguinte (D1, 5ª feira) os voluntários retornavam ao HUOL, quando realizavam nova bateria de exames, para acessar mudanças subagudas. Novamente, os voluntários realizavam avaliação psiquiátrica (MADRS), neuropsicológica, exame de imagem por

[5]Solicitávamos o café da manhã com restrição a café, ovo, e leite.

ressonância magnética (estrutural e funcional), e registro do discurso. Dormiam mais uma noite no laboratório para realização do EEG durante o sono, e na manhã do dia seguinte realizavam nova coleta de sangue e saliva (Fig. 1).

Por volta das 7h30 da 6ª feira (D2) os voluntários respondiam a dois outros questionários (MEQ30 e ARSQ), descreviam novamente a experiência que tiveram durante a sessão de tratamento, e relatavam eventuais mudanças percebidas nos dias que se seguiram a sessão de tratamento. Por volta das 9h, eram avaliados pela equipe de psiquiatria (MADRS), e liberados em seguida.

Os pacientes foram acompanhados no ambulatório de depressão do HUOL para avaliarmos os efeitos antidepressivos de mais longo prazo. Foram agendados retornos nos seguintes momentos pós sessão de tratamento: 7 dias (D7), 14 dias (D14), e a partir daí, a cada mês, por 6 meses. Durante as consultas era realizada avaliação clínica e aplicadas as escalas HAM-D e MADRS. No primeiro retorno do paciente (D7) foi introduzido o novo esquema de tratamento medicamentoso.

4.6. A sessão de tratamento

As sessões de tratamento ocorreram em um ambiente do laboratório de Neuroimagem Funcional (NeuroImago), localizado nas dependências do HUOL, projetado para ser confortável, com características de um quarto de dormir, com cama, poltrona, cortina e quadros. O quarto possui ar-condicionado e iluminação natural, regulada por uma cortina (Fig. 2).

Figura 2 – Ambiente onde ocorreram as sessões de tratamento. O ambiente projetado é confortável, e com características de um quarto de dormir, com cama, poltrona, cortina e quadros. Nesse mesmo quarto eram realizadas as medidas de EEG durante o sono.

A sessão de tratamento ocorria às quartas-feiras, e tinha início por volta das 8h, após a coleta de sangue, do café da manhã, e de um breve descanso. Após breve conversa, iniciá-

vamos a verificação do sistema de EEG, e os eletrodos com valores de impedância superiores a 20 KΩ eram reposicionados. Esse processo incluía limpeza do couro cabeludo sob o eletrodo com álcool, creme esfoliante e preenchimento do espaço entre o eletrodo e o escalpo com gel condutor. Foi feito registro contínuo de eletroencefalograma (EEG), eletrocardiograma (ECG, 2 canais), e eletromiograma (EMG, 3 canais) por um sistema de 32 canais (Brain Products, EasyCap, Herrsching-Breitbrunn, Alemanha), com frequência de amostragem 1 kHz, e o eletrodo FCz como referência.

Por volta das 10h os voluntários eram entrevistados pela equipe de psiquiatria para coletar os dados basais das escalas psiquiátricas (CADSS, BPRS, EAM e MADRS). Logo antes do início da sessão (por volta das 10h30), tínhamos uma conversa final com os voluntários sobre a experiência com a ayahuasca. Reiterávamos que eles poderiam receber tanto placebo como ayahuasca, falávamos sobre a segurança e o gosto forte da bebida, sobre os efeitos mais comuns, como alterações corporais (náusea, vômito e diarreia), alterações na maneira de sentir, pensar, ver e ouvir. Buscávamos deixar claro, porém, que os efeitos eram bastante variados de pessoa para pessoa, e que eles poderiam, inclusive, beber ayahuasca e não sentir nada. Também era reforçado o caráter temporário dos efeitos eventualmente experimentados. Esse conjunto de orientações incluía, ainda, atitudes e estratégias que muitas vezes auxiliam durante a experiência, como a manutenção do foco da atenção na respiração e/ou nas músicas. Nesse momento, também, era sugerido que eles criassem uma *intenção*, que poderia ser um questionamento, um desejo, algo pessoal que eles deveriam recordar ao longo da sessão de tratamento. Esse pedido é comum em diversos rituais que utilizam a ayahuasca, e é considerado parte importante da experiência.

Ao final da conversa era colhida a amostra basal de saliva, para dosagem de cortisol. Logo em seguida, a substância (ayahuasca ou placebo) era administrada. Os voluntários eram orientados a beber toda a quantidade que lhes foi oferecida. Em seguida, oferecíamos um pouco de água e uma pastilha para atenuar o gosto forte da bebida[6].

Após a ingestão, os voluntários permaneciam sentados ou deitados em uma poltrona reclinável confortável (Fig. 2). Eles foram orientados a permanecerem em silêncio, de olhos fechados, mantendo-se concentrados no corpo, pensamentos e emoções. Durante alguns perío-

[6]O placebo também tinha gosto bem forte.

dos pré-estabelecidos eles ouviam música, em outros, ficavam em silêncio. Para a trilha sonora foram escolhidas músicas tocadas nas reuniões da UDV, e incluiu cantores brasileiros como Almir Sater, Alceu Valença, Elba Ramalho, Caetano Veloso, Geraldo Azevedo, Gilberto Gil, Maria Betânia, Renato Teixeira, Rita Lee, Roberto Carlos, Tim Maia, dentre outros. Músicas instrumentais, clássicas e andinas também estavam presentes.

Ao longo de toda sessão, os voluntários foram acompanhados por dois membros da equipe, que ficavam em uma sala contígua ao quarto do voluntário, e que serviam de apoio caso os voluntários sentissem necessidade. A equipe de psiquiatria ficava em outra sala, ao lado, e interrompia a sessão em apenas três momentos pré-determinados: 1h40, 2h40 e 4h após a ingestão. Nesses momentos eram realizadas coletas de saliva e aplicadas as escalas psiquiátricas (CADSS, BPRS, EAM e MADRS).

Logo após a última avaliação psiquiátrica, por volta das 15h (~5h após a ingestão), os voluntários descreviam livremente os efeitos experimentados (registrado em filme) e respondiam à HRS. Por fim, eram avaliados pela equipe de psiquiatria, e liberados para irem à casa[7].

4.7. Análises dos dados

4.7.1. Questionários (HRS e MEQ30)

Utilizamos o teste de Mann-Whitney, para avaliar o efeito do tratamento sobre HRS e a MEQ30. Testes independentes foram realizados para cada um dos fatores dos questionários (6 para a HRS, e 4 para a MEQ30). Foi considerado significativo $p<0,05$ bicaudal, corrigido para múltiplas comparações pelo número de fatores de cada questionário.

4.7.2. Escalas de depressão (HAM-D e MADRS)

Avaliamos o efeito antidepressivo até uma semana após a sessão de tratamento (D7), comparados à linha de base (D-1), a partir dos escores das escalas de depressão HAM-D e MADRS. Utilizamos um modelo geral misto, com os escores basais como covariável, para explorar as alterações nos escores da HAM-D no D7 e nos escores da MADRS no D1, D2 e D7. Uma estrutura de covariância Toeplitz foi o melhor ajuste para os dados de acordo com o

[7]Os voluntários foram orientados a prestar atenção em eventuais mudanças nos dias seguintes à sessão de tratamento. Alguns pacientes, em estado mais grave, permaneceram em um leito da enfermaria de psiquiatria do HUOL durante toda a semana do experimento.

critério de informação de Akaike. Os dados que faltaram foram estimados usando estimativa de máxima verossimilhança restrita (RMLE, do inglês, *restricted maximum likelihood estimation*). Os efeitos principais e a interação tratamento versus tempo foram avaliados. Foram realizados testes t *post-hoc* para comparações entre grupos em todos os pontos (D1, D2 e D7), e o teste de Sidak foi usado para controlar comparações múltiplas. A significância estatística foi avaliada em $p < 0{,}05$, bicaudal.

Os tamanhos de efeitos (Cohen's d) foram obtidos para duas comparações diferentes. Os tamanhos do efeito entre grupos foram calculados as médias estimadas de cada grupo em cada ponto (D1, D2 e D7). Para comparações dentro do grupo, os tamanhos de efeitos de cada tratamento foram calculados separadamente, usando as diferenças entre os valores do ponto e da linha de base.

A resposta clínica foi definida como uma redução de 50% ou mais nos escores basais. A remissão foi definida como HAM-D≤7 ou MADRS≤10. A proporção entre os pacientes que responderam e não responderam e os que ficaram em remissão e não remissão em cada grupo foi estimada utilizando o teste de Fisher. A razão de possibilidades (OR, do inglês, *odds ratio*), e o número necessário para tratar (NNT, do inglês, *number needed to treat*) também foram calculados. Os dados de pacientes que diminuíram os escores da HAM-D ou MADRS em 50% ou mais entre o início do *washout* e a avaliação inicial, ou que estavam em remissão no dia da sessão de tratamento, não foram considerados para análise estatística. Utilizamos o IBM SPSS Statistic 20 e Prism 7 para executar as análises.

4.7.3. Imagem por ressonância magnética

Foram realizados dois exames de imagem por ressonância magnética (MRI, do inglês *Magnetic Resonance Imaging*). O primeiro, no dia anterior (D-1), e o segundo, um dia após a sessão de tratamento (D1). Em cada sessão foram realizados dois protocolos de fMRI: um de regulação emocional e outro durante estado de repouso (*resting state*).

Todas as imagens foram adquiridas em um tomógrafo de imagem por ressonância magnética de 1.5 Tesla (HDxt, General Electric, EUA) que se encontra em atividade regular no centro de diagnóstico por imagem (CDI) do HUOL.

Para o protocolo de regulação emocional e *resting state* foram utilizadas sequências do tipo EPI (do inglês, *Echo Plannar Imaging*), com os seguintes parâmetros: tempo de repeti-

ção (TR) = 2000 ms, tempo ao eco (TE) = 35 ms, ângulo flip = 60 °, FOV = 24 cm, matriz = 64 x 64, número de fatias = 35 fatias, espessura da fatia = 3.0 mm, intervalo entre fatias = 0,3 mm, número de volumes = 165 (regulação emocional), 213 (*resting state*). Imagens estruturais de boa resolução anatômica foram adquiridas utilizando-se sequências FSPGR BRAVO com os seguintes parâmetros: TR = 12.7 ms, TE = 5.3 ms, flip = 60 °, FOV = 26 cm, matriz = 320 x 320; número de fatias = 128, espessura da fatia = 1.0 mm; número de volumes = 1.

Os estímulos foram apresentados por retroprojeção em tela de acrílico translúcida posicionada no interior da sala do scanner, aos pés do voluntário. O programa Psychopy (versão 1.79.00) foi utilizado para controlar a apresentação de estímulos e coletar as respostas. Foram fornecidas lentes de contato para os voluntários que apresentavam acuidade visual reduzida, que dificultasse a visualização das imagens.

Antes do experimento, todos os participantes praticaram o protocolo de regulação emocional em um computador até que estivessem seguros da tarefa. Uma vez posicionados no scanner, tinham a oportunidade de treinar por mais alguns minutos, até se sentirem confortáveis com o novo ambiente. Um botão de respostas de fibra ótica (Pyka com 905 interface da Current Designs, Philadelphia, USA) foi utilizado para obter as respostas durante o julgamento das imagens.

Durante o protocolo de regulação emocional, adaptado de (Ochsner et al. 2002) os voluntários observavam uma série de imagens com conteúdo emocional, extraídas do banco de imagens IAPS (do inglês, *International Affective Picture System*) (Lang, Bradley, and Cuthbert 2008). Foram selecionadas 72 imagens com conteúdo emocional negativo e 36 neutras, classificadas com base em escores de valência e excitação *(arousal)*. As imagens negativas continham baixa valência (2,44 ± 0,61) e alta excitação (5,77 ± 0,79). As neutras eram de valência média (5,14 ± 0,36) e excitação baixa (3,13 ± 0,57).

Três condições foram examinadas: i) olhar passivamente imagens neutras (NEU), ii) olhar passivamente imagens negativas (NEG), iii) reavaliar (*reappraise*) imagens negativas (REAP). Nas condições i) e ii) os voluntários deveriam observar a imagem, e em seguida julgar seu impacto emocional utilizando uma escala *Likert* de cinco pontos[8]. Na condição iii) o

[8] (1) muito negativa, (2) negativa, (3) neutra, (4) positiva ou (5) muito positiva

indivíduo deveria tentar regular o impacto emocional das imagens, a partir reinterpretação (reavaliação) das imagens, tentando torná-las menos negativas. Algumas estratégias de reavaliação incluíam pensar que a "imagem não é verdadeira", que "é a cena de um filme", que "a pessoa da cena é um dublê." Pedíamos explicitamente que evitassem fechar os olhos ou desviar o olhar da imagem.

Durante cada sessão (antes e após a sessão) foram adquiridos 3 *runs* (~5,5 minutos cada). Cada *run* continha 18 *trials* (uma imagem por *trial*), sendo 6 *trials* por condição (NEU, NEG, REAP), balanceados por tipo de imagem (neutra ou negativa) e instrução (olhar ou regular).

A estrutura de cada *trial* é representada na figura 3. Cada *trial* teve duração de **18** s divididos da seguinte forma: **2** s da instrução (olhe a imagem ou torne a imagem positiva), **6** s de apresentação da imagem (neutra ou negativa), **6** s para realizar o julgamento, **4** s de apresentação de uma cruz de fixação, que serviu de linha de base (*baseline*).

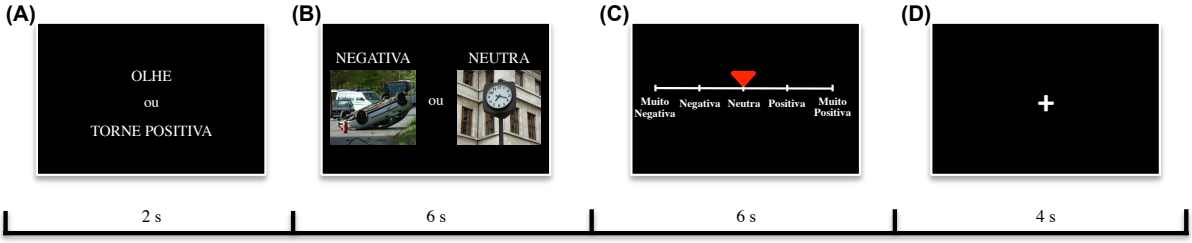

Figura 3 – Representação de um *trial* do protocolo de regulação emocional. (A) No início, por 2 s, era apresentada a instrução (olhe a imagem ou torne a imagem positiva). **(B)** Apresentação da imagem (neutra ou negativa) por 6 s **(C)** Avaliação do impacto emocional da imagem por 6 s. **(D)** Apresentação de uma cruz de fixação (*baseline*) por 4 s. Esse bloco (A + B + C + D) foi repetido 18 vezes, sendo seis vezes para cada condição (NEU, NEG, REAP). A duração total de cada *run* foi de 324 s (~5,5 minutos). Em cada sessão (antes e após o tratamento) foram realizados 3 *runs* de 18 *trials* cada.

Durante todo protocolo de *resting state* (7,1 minutos) os voluntários deveriam permanecer deitados, imóveis e de olhos fechados. Os voluntários foram orientados a prestarem atenção aos seus pensamentos durante esse período, pois teriam que relatá-los ao término do exame.

Análise estatística do protocolo de regulação emocional

Realizamos a análise dos dados do protocolo de regulação emocional no programa SPM12 (*Statistical Parametric Mapping, Wellcome Institute of Cognitive Neurology*, Londres, Inglaterra). Os 3 primeiros volumes (*dummy scans*) foram excluídos para permitir o

equilíbrio em T1. A etapa de pré-processamento incluiu: correção de movimento, correção de tempo entre fatias, aplicação de filtros espacial gaussiano (FWHM=8 mm) e temporal (passa-alta em 0.1 Hz), e normalização das imagens estruturais e EPI para o padrão MNI152 (*Montreal Neurological Institute*), de 2 mm^3 de resolução espacial.

Para melhorar a qualidade da correção de movimento, utilizamos ferramentas de detecção de artefato (ART)[9] com o qual o movimento composto é calculado. Essa medida é um índice (FD, do inglês, *frame displacement*) que compreende o movimento de rotação (α, β e γ) e translação entre volumes (x, y e z):

$$FD_i = |\Delta d_{xi}| + |\Delta d_{yi}| + |\Delta d_{zi}| + |\Delta \alpha_i| + |\Delta \beta_i| + |\Delta \gamma_i|,$$

Em que $\Delta d_{ix} = d_{(i-1)x} - d_{ix}$, e de maneira similar para os demais parâmetros. Os parâmetros de rotação foram convertidos de graus para milímetros calculando o deslocamento na superfície de uma esfera de raio de 50 mm, que é aproximadamente a distância média do córtex cerebral para o centro da cabeça (Power et al. 2012).

Todos os volumes com movimento composto superior a 1 mm ou a 3 desvios-padrão (DP) da média do sinal global (em um *run*) foram considerados *outliers*. Em média, 4% dos volumes por voluntário controle e 7,5% dos volumes por paciente foram detectados como *outliers*. Foram excluídos *runs* em que mais de 20% do total de volumes foi detectado como *outlier*.

Para cada sujeito, um modelo linear geral (GLM, do inglês, *General Linear Model*) foi utilizado para estimar a resposta hemodinâmica em cada condição. Cinco condições foram modeladas: instrução, olhar imagem neutra (NEU), olhar imagem negativa (NEG), regular (REAP) e julgar. Cada uma delas foi modelada pela convolução de uma função retangular, que simula os períodos de cada condição, com uma função de resposta hemodinâmica canônica. Adicionalmente, alguns regressores de não interesse foram incluídos, entre eles os seis parâmetros de movimento de cabeça (3 de translação e 3 de rotação) e um regressor para cada volume considerado *outlier*. Correlações seriais foram corrigidas utilizando-se o modelo autoregressivo AR(1).

Para cada participante uma imagem contraste foi criada, uma para cada condição de interesse: **(i)** olhar imagem negativa > olhar imagem neutra (NEG > NEU), **(ii)** tornar ima-

[9]http://www.nitrc.org/projects/artifact_detect/

gem positiva > olhar imagem neutra (REAP > NEU), **(iii)** tornar imagem positiva > olhar imagem negativa (REAP > NEG). Essas imagens de contraste foram utilizadas na análise de segundo nível, descrita a seguir.

Para investigar o efeito da ayahuasca nos mecanismos de regulação emocional, utilizamos análise de variância (ANOVA) de medidas repetidas com 2 fatores, tratamento (ayahuasca ou placebo) e tempo (pré e pós tratamento) em uma análise exploratória no cérebro inteiro *(whole brain)* nos três contrastes de interesse: NEG > NEU, REAP > NEU, REAP > NEG. O limiar estatístico considerado foi $p < 0{,}001$ para definição dos clusters (CDT, do inglês *cluster defining threshold*) e $p_{\text{cluster FWE}} < 0{,}05$ (Eklund, Nichols, and Knutsson 2016).

Além disso, foram realizadas análises em regiões de interesse (ROI) definidas a partir de trabalhos prévios sobre regulação emocional (Hermann et al. 2014, Ochsner, Silvers, and Buhle 2012, Ochsner et al. 2002). As seguintes ROIs foram selecionadas (Fig. 4): córtex pré-frontal dorsolateral (dLPFC), córtex pré-frontal dorsomedial (dmPFC), córtex pré-frontal ventromedial (vmPFC), córtex pré-frontal ventrolateral (vlPFC), amígdala (amygdala), ínsula anterior (antInsula), núcleo accumbens (NAc), córtex órbitofrontal (OFC), cíngulo anterior dorsal (dACC) e cíngulo anterior subgenual (sgACC).

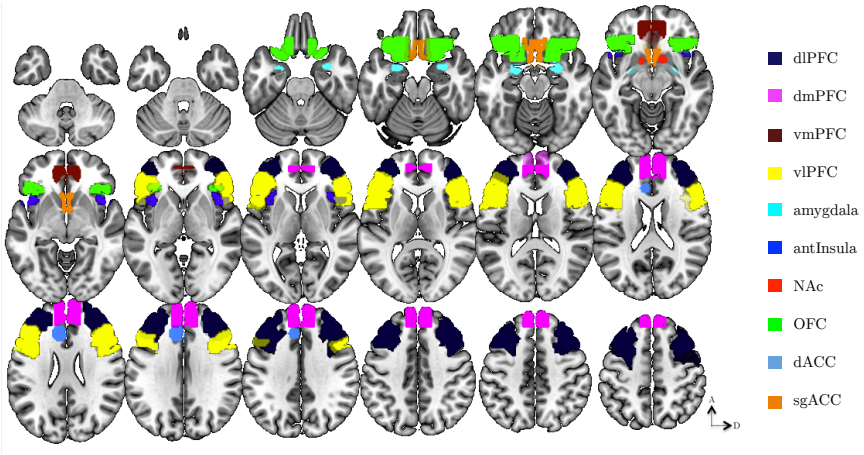

Figura 4 – Regiões de interesse (ROI) utilizadas para análise do protocolo de regulação emocional**.** As seguintes regiões foram selecionadas: córtex pré-frontal dorsolateral (dlPFC), córtex pré-frontal dorsomedial (dmPFC), córtex pré-frontal ventromedial (vmPFC), córtex pré-frontal ventrolateral (vlPFC), amígdala (amygdala), ínsula anterior (antInsula), núcleo accumbens (NAc), córtex órbitofrontal (OFC), cíngulo anterior dorsal (dACC) e cíngulo anterior subgenual (sgACC). A = anterior, D = direita.

As máscaras do dlPFC, vlPFC, dlPFC e vmPFC foram cedidas por Morris e colegas (Morris et al. 2016). As máscaras da amígdala, núcleo accumbens e córtex órbitofrontal foram obtidas diretamente no atlas WFU PickAtlas (Maldjian et al. 2003) do SPM12. A más-

cara da ínsula anterior foi obtida de um atlas funcional (Shirer et al. 2012). A máscara do dACC foi formada por uma esfera de 10 mm de raio centrada nas seguintes coordenadas MNI: x = -8, y = 28, z = 28 (Hermann et al. 2014). E o cíngulo anterior subgenual foi baseado na área de Brodmann 25 e no córtex subcaloso do atlas Harvard-Oxford do FSL (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/Atlases). A figura 4 ilustra as máscaras utilizadas na análise por ROI.

Resultados

ARTIGO I

Rapid antidepressant effects of the psychedelic ayahuasca in treatment-resistant depression: a randomized placebo-controlled trial

Fernanda Palhano-Fontes, Dayanna Barreto, Heloisa Onias, Katia C Andrade, Morgana Novaes, Jessica A Pessoa, Sergio A Mota-Rolim, Flavia Osório, Rafael Sanches, Rafael G dos Santos, Luís F Tófoli, Gabriela de Oliveira Silveira, Mauricio Yonamine, Jordi Riba, Francisco RR Santos, Antonio A Silva-Junior, João Alchieri, Nicole L Galvão-Coelho, Bruno Lobão-Soares, Jaime Hallak, Emerson Arcoverde, João P Maia-de-Oliveira, Dráulio B Araújo

(Artigo submetido)

Rapid antidepressant effects of the psychedelic ayahuasca in treatment-resistant depression: a randomized placebo-controlled trial

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Abstract

Recent open label trials show that psychedelics, such as ayahuasca, hold promise as fast-onset antidepressants in treatment-resistant depression. In order to further test the antidepressant effects of ayahuasca, we conducted a parallel-arm, double-blind randomized placebo-controlled trial in 29 patients with treatment-resistant depression. Patients received a single dose of either ayahuasca or placebo. Changes in depression severity were assessed with the Montgomery–Åsberg Depression Rating Scale (MADRS) and the Hamilton Depression Rating scale (HAM-D). Assessments were made at baseline, and at one (D1), two (D2) and seven (D7) days after dosing. We observed significant antidepressant effects of ayahuasca when compared to placebo at all timepoints. MADRS scores were significantly lower in the ayahuasca group compared to placebo (at D1 and D2: $p=0.04$; and at D7: $p<0.0001$). Between-group effect sizes increased from D1 to D7 (D1: Cohen's $d=0.84$; D2: Cohen's $d=0.84$; D7: Cohen's $d=1.49$). Response rates were high for both groups at D1 and D2, and significantly higher in the ayahuasca group at D7 (64% vs. 27%; $p=0.04$), while remission rate was marginally significant at D7 (36% vs. 7%, $p=0.054$). To our knowledge, this is the first controlled trial to test a psychedelic substance in treatment-resistant depression. Overall, this study brings new evidence supporting the safety and therapeutic value of ayahuasca, dosed within an appropriate setting, to help treat depression.

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Introduction

The World Health Organization estimates that 350 million people suffer from depression, and about one-third do not respond to appropriate courses of at least three antidepressants.[1–3] Most currently available antidepressants have a similar efficacy profile and mechanisms of action, based on the modulation of brain monoamines, and take about two weeks to start being effective.[1–3]

Recent evidence, however, shows a rapid and significant antidepressant effect of ketamine, an N-methyl-D-aspartate (NMDA) antagonist frequently used in anesthesia.[4–7] Namely, in randomized placebo-controlled trials, the antidepressant effects of ketamine in treatment-resistant depression peaked one day after dosing and remained significant for about seven days.[4–7]

Additionally, research with serotonergic psychedelics has gained momentum.[8] A few centers around the world are currently exploring how these substances affect the brain, and also probing their potential in treating different psychiatric conditions, including mood disorders.[9–13] For instance, recent open label trials show that psychedelics, such as ayahuasca and psilocybin, hold promise as fast-onset antidepressants in treatment-resistant patients.[10,13] Moreover, randomized controlled trials have recently shown that psilocybin reduces anxiety and depression symptoms in patients with life-threatening cancer.[11,12]

Ayahuasca is a brew traditionally used for healing and spiritual purposes by indigenous populations of the Amazon Basin. In the 1930s, it began to be used in religious settings of Brazilian small urban centers, reaching large cities in the 1980s and expanding since then to several other parts of the world.[14] In Brazil, ayahuasca has a legal status for ritual use since 1987. Ayahuasca is prepared by decoction of two plants: *Psychotria viridis* that contains the psychedelic N,N-dimethyltryptamine (N,N-DMT), a serotonin and sigma-1 receptors agonist,[15] and *Banisteriopsis caapi*, rich in reversible monoamine oxidase inhibitors such as harmine, harmaline, and tetrahydroharmine.[16]

The acute psychological effects of ayahuasca last around 4h and include intense perceptual, cognitive and affective changes.[16–18] Although nausea, vomiting and diarrhea are often reported, mounting evidence points to a positive safety profile of ayahuasca. For instance, ayahuasca is not addictive and has not been associated with psychopathological, personality or cognitive deterioration, and it promotes only moderate sympathomimetic effects.[19–21] The main concern is rare instances of prolonged increases in psychotomimetic symptoms, especially in individuals prone to psychosis.[18,22]

In a recent open label trial, 17 patients with major depressive disorder attended a single dosing session with ayahuasca. Depression severity was assessed before, during and after dosing by the Hamilton Depression Rating Scale (HAM-D) and the Montgomery–Åsberg Depression Rating Scale (MADRS).[10] Significant reduction in depression severity was found already in the first hours after dosing, an effect that remained significant for 21 days.[10,23]

Although promising, these studies have not controlled for the placebo effect, which can be remarkably high in clinical trials for depression, reaching 30-40% of the patients.[24] To address this issue, and to further test the antidepressant effects of ayahuasca, we conducted a placebo-controlled trial in patients with treatment-resistant major depression.

Materials and Methods

Study design and participants

This study is a double-blind parallel-arm randomized placebo-controlled trial. Patients were recruited from psychiatrist referrals from local outpatient psychiatric units or through media advertisements. All procedures took place at the Onofre Lopes University Hospital (HUOL), Natal-RN, Brazil. The University Hospital Research Ethics Committee approved the study (# 579.479), and all subjects provided written informed consent before participation.

We recruited adults aged 18-60 years who met criteria for unipolar major depressive disorder as diagnosed by the Structured Clinical Interview for Axis I (DSM- IV). Only treatment-resistant patients were selected, defined herein as those with inadequate responses to at least two antidepressant medications from different classes.[2] Selected patients were in a current depressive episode of moderate-to-severe at screening (HAM-D≥17). We adopted the following exclu-

sion criteria: previous experience with ayahuasca, current medical disease based on history, pregnancy, current or previous history of neurological disorders, history of schizophrenia or bipolar affective disorder, history of mania or hypomania, use of substances of abuse, and suicidal risk.

Randomization and masking

Patients were randomly assigned (1:1) to receive ayahuasca or placebo, using permuted blocks of size 10. All investigators and patients were blind to intervention assignment, which was kept only in the database and with the pharmacy administrators. Masking was further achieved by ensuring that all patients were naïve to ayahuasca, and by randomly assigning a different psychiatrist for patient follow-up assessments. Both ayahuasca and placebo were kept in identical amber glass bottles.

Procedures

The substance used as placebo was a liquid designed to simulate the organoleptic properties of ayahuasca, such as a bitter and sour taste, and a brownish color. It contained water, yeast, citric acid, zinc sulphate and a caramel colorant. The presence of zinc sulphate also produced low to modest gastrointestinal distress. A single ayahuasca batch was used throughout the study. The batch was prepared and provided free of charge by a branch of the Barquinha church based in the city of Ji-Paraná-RO, Brazil.

To assess alkaloids concentrations and stability of the batch, samples of ayahuasca were quantified at two different moments by mass spectroscopy analysis (details in suppl. material). On average, the ayahuasca contained (mean±SD): 0.36±0.01 mg/mL of N,N-DMT, 1.86±0.11 mg/mL of harmine, 0.24±0.03 mg/mL of harmaline, and 1.20±0.05 mg/mL of tetrahydroharmine. Data from the individual assessments are shown in supplementary table S1.

After screening, patients underwent a washout period adjusted to the half-life time of their current antidepressant medication, which typically lasted two weeks. During the dosing session, patients were not under any antidepressant medication, and a new treatment scheme was introduced only seven days after dosing. If needed, benzodiazepines were allowed as a supporting hypnotic and/or anxiolytic agents (suppl. table S2).

Two clinical scales for depression assessed changes in depression severity: MADRS and HAM-D. Assessments were made at baseline (one day before dosing), and at one (D1), two (D2) and seven (D7) days after dosing. HAM-D was applied only at baseline and D7, as it was designed to access depression symptoms present in the last week.[25]

Dosing sessions lasted approximately 8 hours, from 8:00 to 16:00, and intake usually occurred at 10:00. After a light breakfast, patients were reminded about the effects they could experience, and strategies to help alleviating eventual difficulties. Sessions took place in a quiet and comfortable living room-like environment, with a bed, a recliner, controlled temperature, natural and dimmed light.

Patients received a single dose of 1 ml/kg of placebo or ayahuasca adjusted to contain 0.36 mg/kg of N,N-DMT. They were asked to remain quiet, with their eyes closed, while focusing on their body, thoughts and emotions. They were also allowed to listen to a predefined music playlist. Patients received support throughout the session from at least two investigators who remained in a room next door, offering assistance when needed. Acute effects were assessed with the Clinician-Administered Dissociative States Scale (CADSS) and the Brief Psychiatric Rating Scale (BPRS), which were applied at -10 min, +1:40h, +2:40h and +4:00h after intake.

When acute psychedelic effects ceased, patients debriefed their experience, and had a final psychiatric evaluation. Around 16:00 they could go home accompanied by a relative or friend. Patients were asked to return for follow-up assessments one, two and seven days after dosing, when also a new antidepressant was prescribed.

Outcomes

The primary outcome measure was the change in depression severity assessed by the HAM-D scale, comparing baseline with D7. In this study we chose D7 as the primary assessment point to allow direct comparison with previous randomized trials of ketamine in treatment-resistant depression.[4–7] Furthermore, D7 was chosen to avoid interaction with the new antidepres-

sant medication, which was prescribed also at D7. The secondary outcome was the change in MADRS scores from baseline to D1, D2 and D7. We examined the proportion of patients meeting response criteria, defined as a reduction of 50% or more in baseline scores. Remission rates, defined as HAM-D≤7 or MADRS≤10, were also examined. Both response and remission rates were computed using scores from HAM-D (at D7) and MADRS (at D1, D2, and D7). Safety and tolerability was also assessed by evaluation of dissociative and psychotomimetic symptoms using the CADSS and BPRS–Positive Subscale (BPRS+) during the dosing session.

Statistical analysis

Analyses adhered to a modified intent-to-treat principle, including all patients who completed assessments at baseline, dosing and D7. An estimated sample size of 42 patients is needed to provide 80% power to detect a 5-point HAM-D difference (standardized effect size=0.9) for differences of ayahuasca and placebo between baseline and D7 with two-sided 5% significance. A fixed-effects linear mixed model, with baseline scores as covariate, examined changes in HAM-D scores at D7, and in MADRS scores at D1, D2 and D7. A Toeplitz covariance structure was the best fit to the data according to Akaike's information criterion. Missing data were estimated using restricted maximum likelihood estimation. Main effects and treatment vs. time interaction were evaluated. Post-hoc t-tests were performed for between-groups comparisons at all timepoints, and Sidak's test was used to control for multiple comparisons. Significance was evaluated at $p<0.05$, two-tailed. Cohen's d effect sizes were obtained for between and with-in group comparisons. Between-group effect sizes were calculated using the estimated means of each group at each timepoint. For within-group comparisons, effect sizes of each treatment were calculated separately, using the differences between an endpoint and baseline values. Differences in proportion of responders/non-responders and remitters/non-remitters between treatments were estimated using Fisher's exact test. Odds ratio (OR) and number needed to treat (NNT) were also calculated. Data from patients whose HAM-D or MADRS scores reduced by 50% or more between washout onset and baseline assessment, or were in remission in the day of dosing, were not considered for statistical analysis. We used IBM SPSS Statistic 20 and Prism 7 to run the analyses. This study is registered with http://clinicaltrials.gov (NCT02914769).

Results

From January 2014 to June 2016, we assessed 218 patients for eligibility, and 35 met criteria for the trial. Six volunteers had to be excluded: five no longer met criteria for depression in the day of dosing, and one dropped out before dosing. Data from 29 patients were included in the analysis: 14 in the ayahuasca group and 15 in the placebo group. Figure 1 shows the trial profile.

On average, patients met criteria for moderate-to-severe depression (mean±SD): HAM-D=21.83±5.35; MADRS=33.03±6.49. They had been experiencing depressive symptoms for 11.03±9.70 years, and had tried 3.86±1.66 different previous unsuccessful antidepressants. Two patients had previous history of electroconvulsive therapy (ECT). Most patients (76%) had a comorbid personality disorder, and 31% had comorbid anxiety disorder. All patients were under regular use of benzodiazepines during the trial (suppl. table S2).

Demographic and clinical characteristics are summarized in table 1 (suppl. table S2 for individual data). All patients were Brazilian, mostly female (72%) adults (42.03±11.66 yo) from low socioeconomic status backgrounds: low educated (41% with <8 years of formal education) and living in low household income (41% earn <2 minimum wages).

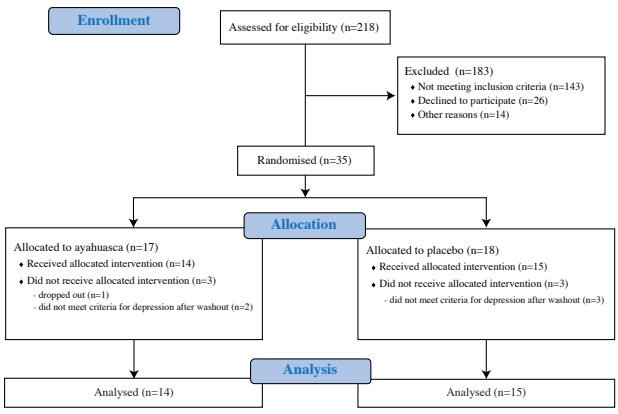

Figure 1. Trial profile.

Figure 2 shows changes in HAM-D scores from baseline to seven days after dosing. We observe a significant between-groups difference at D7 (F_1=6.31; p=0.019) with patients treated with ayahuasca presenting less severity when compared to patients treated with placebo (suppl. figure S1 for individual scores). Between-group effect size is large at D7 (Cohen's d=0.98; CI 95%: 0.21 to 1.75). Within-group effect size (suppl. table S3) is large for the ayahuasca group (Cohen's d=2.22; CI 95%: 1.28 to 3.17), and medium for the placebo (Cohen's d=0.46; CI 95%: -0.27 to 1.18).

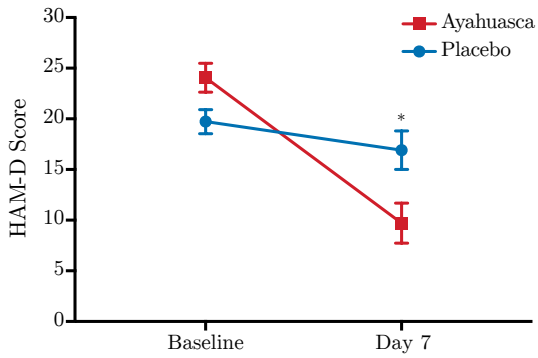

Figure 2. HAM-D scores at baseline and seven days after dosing. Statistical analysis shows a significant difference between ayahuasca (red squares) and placebo (blue circles) seven days after dosing (p=0.019). Between-group effect size is high (Cohen's d=0.98). Values are (mean ± SEM). HAM-D scores: mild depression (8–16), moderate (17–23), severe (≥ 24).

Figure 3 shows mean MADRS scores as a function of time. Linear mixed model shows a significant effect for time ($F_{2,34.4}$=3.96; p=0.028), treatment ($F_{1,27.7}$=10.52; p=0.003), but no treatment vs. time interaction ($F_{2,34.4}$=1.77; p=0.185). Individual scores are presented in supplementary figure S2. Post-hoc analysis shows a significant difference between groups at D1 ($F_{1,49.7}$=4.58; p=0.04), D2 ($F_{1,50.3}$=4.67; p=0.04) and D7 ($F_{1,47}$=14.81; p<0.0001).

Between-groups effect size is large at D1 (Cohen's d=0.84; CI 95%: 0.05 to 1.62) and D2 (Cohen's d=0.84; CI 95%: 0.05 to 1.63) and largest at D7 (Cohen's d=1.49; CI 95%: 0.67 to 2.32).

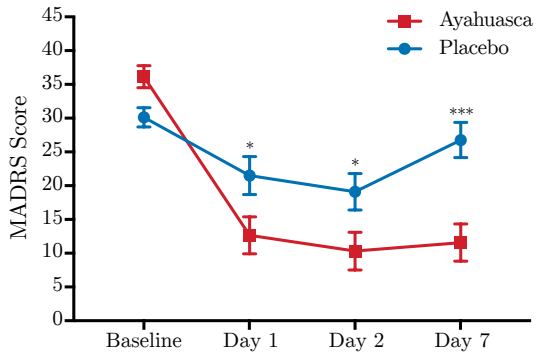

Figure 3. MADRS scores as a function of time. Significant differences are observed between ayahuasca (red squares) and placebo (blue circles) at D1 (p=0.04), D2 (p=0.04) and D7 (p<0.0001). Between groups effect sizes are high at all timepoints after dosing: D1 (Cohen's d=0.84), D2 (Cohen's d=0.84) and D7 (Cohen's d=1.49). Values are (mean ± SEM). MADRS scores: mild depression (11–19), moderate (20–34), severe (≥ 35). *p <0.05; ***p <0.0001.

Within-group effect sizes (suppl. table S4) are large for the ayahuasca group at all timepoints after dosing (Cohen's d=2.78, CI 95%: 1.74 to 3.82 at D1, 3.05, CI 95%: 1.94 to 4.16 at D2, 2.90, CI 95%: 1.84 to 3.97 at D7).

HAM-D response rate at D7 was significantly different between-groups, with 57% of responders in the ayahuasca group against 20% in the placebo group (OR=5.33 [95% CI: 1.11 to 22.58]; p=0.04; NNT=2.69). The difference HAM-D remission rate was marginally significant at D7: 43% in ayahuasca vs. 13% in placebo (OR=4.87 [95% CI: 0.77 to 26.73]; p=0.07; NNT=3.39).

Figure 4a shows the MADRS response rates as a function of time. At D1, response rates were high for both groups: 50% in the ayahuasca group, and 46% in the placebo group (OR=1.17 [95% CI: 0.26 to 5.48]; p=0.87; NNT=26). At D2, they remained high in both groups: 77% in the ayahuasca group and 64% in the placebo (OR=1.85 [95% CI: 0.29 to 8.40]; p=0.43; NNT=7.91). Response rate was statistically different at D7: 64% of responders in the ayahuasca group, and 27% in the placebo (OR=4.95 [95% CI: 1.11 to 21.02]; p=0.04; NNT=2.66).

Table 1. Socio-demographic & clinical characteristics

	Ayahuasca	Placebo
Participants, n	14	15
Age (years)	39.71±11.26	44.2±11.98
Gender (M/F)	3/11	5/10
Unemployed (%)	7/14 (50)	8/15 (53)
Household income		
< 2 minimum wages (%)	6/14 (43)	6/15 (40)
2-5 wages (%)	4/14 (28)	7/15 (47)
6-10 wages (%)	1/14(7)	1/15 (6.6)
11 or more wages (%)	3/14 (21)	1/15 (6.6)
Education		
Up to 8 years, n (%)	6/14 (43)	6/15 (40)
9-11 years, n (%)	3/14 (21)	5/15 (33)
12-16 years, n (%)	2/14(14)	2/15 (13)
17 or more years, n (%)	3/14 (21)	2/15 (13)
Religion (%)		
Catholic	7/14 (50)	5/15 (33)
Protestant	4/14 (28)	1/15 (6.6)
Other	0/14 (0)	4/15 (27)
No religion	3/14 (21)	5/15 (33)
Ethnicity (%)		
Caucasian	9/14 (64)	8/15 (54)
Black	1/14 (7)	0/15 (0)
Pardo	4/14 (28)	7/15 (47)
Clinical characteristics		
Age of depression onset (years)	30.93±10.19	30.87±13.39
Illness duration (years)	8.78±6.25	13.13±11.92
Number of previous episodes	2.71±1.32	3.53±1.76
Length of current episode (months)	14.71±18.92	10.13±9.15
Failed antidepressant medications	3.93±1.44	3.8±1.89
History of ECT (%)	1/14 (7)	1/15 (6.6)
History of psychotherapy (%)	11/14 (79)	12/15 (80)
Anxiety disorder (%)	5/14 (36)	5/15 (33)
Personality disorder (%)	10/14 (71)	12/15 (80)
Melancholic (%)	12/14 (83)	12/15 (80)
Atypical (%)	2/14 (14)	3/15 (20)
Baseline HAM-D	24.07±5.34	19.73±4.59
Baseline MADRS	36.14±6.12	30.13±5.55

Values are (mean ± SD); M=male; F=female; ECT=electroconvulsive therapy.

Figure 4b shows the MADRS remission rates as a function of time. At D1 and D2, remission rates were not statistically different between groups (p=0.86 and p=0.31, respectively). At D7 MADRS remission rate was marginally significant: 36% of remitters in the ayahuasca group and 7% in the placebo (OR=7.78 [95% CI: 0.81 to 77.48]; p=0.054; NNT=3.44). Supplementary figure S2 shows individual MADRS scores %-changes from baseline, at all timepoints. Although individual variance is high, overall, we find decreased scores for all subjects in the ayahuasca group.

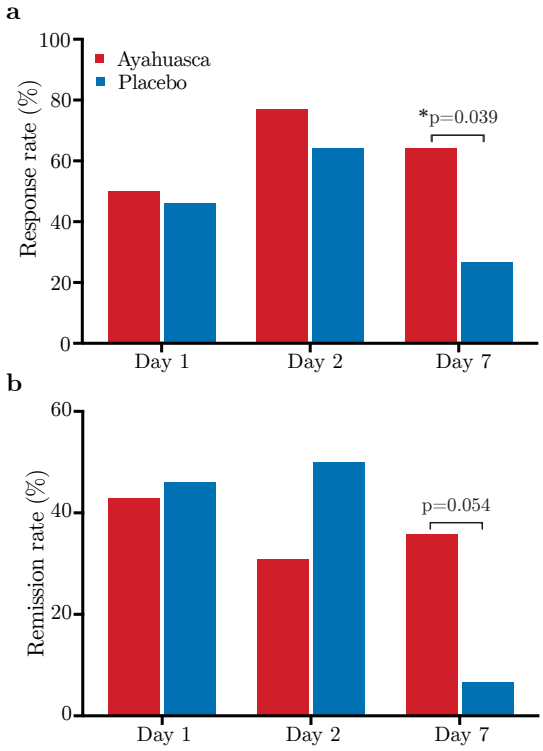

Figure 2. Response and remission rates as a function of time. Response **(a)** and remission **(b)** rates were high for both groups at D1 and D2. At D7, responses rate is significantly higher for ayahuasca (OR=4.95 [95% CI: 1.11 to 21.02]; p=0.04; NNT=2.66), while remission rate is marginally significant (OR=7.78 [95% CI: 0.81 to 77.48]; p=0.054; NNT=3.44).

The most frequently observed adverse effects in the ayahuasca group included nausea (71%), vomiting (57%), transient anxiety (50%), transient headache (42%), and restlessness (50%) (suppl. table S5). Patients exhibited transient dissociative and psychotomimetic symptoms as measured by CADSS and BPRS+ scales, with slightly increased scores +1h40 after ayahuasca intake: 34.8% (BPRS+) and 21.6% (CADSS) (suppl. table S6).

Discussion

We found evidence for rapid antidepressant effects after a single dosing session with ayahuasca, when compared to placebo. Depression severity changed significantly but differently for the ayahuasca and placebo groups. At all timepoints before dosing, improvements in the psychiatric scales observed in the ayahuasca group were significantly higher than those of the placebo group, with increasing between-group effect sizes from D1 to D7. Response rates were high for both groups at D1 and D2, and were significantly higher in the ayahuasca group at D7. Remission rate was marginally significant at D7.

The within-group effect size found for ayahuasca at D7 (Cohen's d=2.22) is compatible with our earlier open label study (Cohen's d at D7=1.83),[10] and compatible with the one found in a recent open label trial with psilocybin for depression (Hedges' g=3.1).[13]

Our results are comparable with randomized controlled trials that used ketamine in treatment-resistant depression.[4–7] Although both ketamine and ayahuasca are associated with rapid antidepressant effects, their response time-courses and mechanisms of action seem to differ. Previous studies with ketamine have found the largest between-group effect size at D1 (Cohen's d=0.89), reducing towards D7 (Cohen's d=0.41).[4–7] In contrast, the effect sizes observed herein were smallest at D1 (Cohen's d=0.84), and largest at D7 (Cohen's d=1.49). These differences are also reflected in the response rate. At D1, the response rate to ketamine lies between 37-70%,[4–7] whereas in our study 50% of the patients responded to ayahuasca. At D7, the ketamine response rate ranges between 7-35%,[4–7] while in our study 64% responded to ayahuasca.

The placebo effect was high in our study, and higher than most studies with ketamine.[4–7] While we find a response rate to placebo of 46% at D1, and 26% at D7, ketamine trials have found a placebo effect on the order of 0-6% at D1, and 0-11% at D7.[4–7] Several factors may contribute to the high placebo effects observed here. First, higher placebo effects are found in patients with low socioeconomic status,[24] which was the

case of our study, where most of the patients were living under significant psychosocial stressors. During our trial, on the other hand, they stayed at a very comfortable and very supportive environment. It is more of a caring effect, than properly a placebo effect. Second, patients with comorbid personality disorders present higher placebo responses.[25] In our study, most patients (76%) also suffered from personality disorders, most of them in cluster B.

A growing body of evidence gives support to the observed rapid antidepressant effects of ayahuasca. For instance, the sigma-1 receptor (σ1R) has recently been implicated in depression, and was reported to be activated by N,N-DMT.[1,15] Moreover, it has been shown that the administration of σ1R agonists results in antidepressant-like effects, which are blocked by σ1R antagonism.[1] Furthermore, σ1R upregulates neurotrophic factors such as brain derived neurotrophic factor (BDNF) and nerve growth factor (NGF), proteins whose regulation and expression seem to be involved in the pathophysiology of depression.[1]

Studies in animal models reported that chronic administration of harmine reduces immobility time, increases climbing and swimming time, reverses anhedonia, increases adrenal gland weight, and increases BDNF levels in the hippocampus.[26,27] All of these are compatible with antidepressant effects. Likewise, harmine seems to stimulate neurogenesis of human neural progenitor cells, derived from pluripotent stem cells, a mechanism also observed in rodents following antidepressant treatment.[28] Also, a recent study in rodents found that a single ayahuasca dose increases swimming time in a forced-swim test.[29]

Over the last two decades, mental health evaluations of regular ayahuasca consumers have shown preserved cognitive function, increased well-being, reduction of anxiety, and depressive symptoms when compared to non-ayahuasca consumers.[19,20] Moreover, a recent study observed that a single dose of ayahuasca enhanced mindfulness-related capacities,[30] and meditation practices have been associated with antidepressant effects.[31]

Brain circuits modulated by psychedelics show great overlap with those involved in mood disorders.[8] We recently found that a single ayahuasca session in patients with depression increases blood flow in brain regions consistently implicated in the regulation of mood and emotions,[3] such as the left nucleus accumbens, right insula and left subgenual area.[10] Moreover, we have shown that ayahuasca reduces the activity of the Default Mode Network (DMN),[32] a brain network found to be hyperactive in depression, possibly due to rumination.[3,33]

No serious adverse effects were observed during or after the dosing session. Although 100% of the patients reported feeling safe during the ayahuasca session, it was not necessarily a pleasant experience. In fact, some patients reported the opposite, as the experience was accompanied by much psychological distress. Most patients reported nausea, and about 57% have vomited, although vomiting is traditionally not considered a side effect of ayahuasca, but rather part of a purging process.[17]

Although promising, this study has some caveats and limitations worth mentioning. The number of participants is modest, and therefore randomized trials in larger populations are necessary. The study was limited to patients with treatment-resistant depression, with long course of illness, and high comorbid personality disorder, which altogether precludes a simple extension of these results to other classes of depression. Another challenge of the research with psychedelics is maintaining double blindness, as the effects of psychedelics are unique. We were particularly keen to ensure blindness throughout the entire experiment, and to that end we adopted a series of additional measures to preserve blindness. All patients were naïve to ayahuasca, with no previous experience with any other psychedelic substance. Clinical evaluations involved a team of five psychiatrists. For every patient, one psychiatrist was responsible for clinical evaluation during the dosing session, and another for the follow-up assessments. The substance used as placebo increased anxiety and induced nausea. In fact, five patients misclassified placebo as ayahuasca, and two of them showed response at D7. Therefore, we believe blindness was adequately preserved in our study.

Since the prohibition of psychedelics in the late 1960s, research with these substances has almost come to a halt. Before research re-

strictions, psychedelics were at early stage testing for many psychiatric conditions, including obsessive-compulsive disorder and alcohol dependence. By mid 1960s, over 40.000 subjects had participated in clinical research with psychedelics, most of them in uncontrolled settings.[8] To our knowledge, this is the first randomized placebo-controlled trial to investigate the antidepressant potential of a psychedelic in a population of patients with treatment-resistant depression. Overall, this study brings new evidence supporting the safety and therapeutic value of psychedelics, dosed within an appropriate setting, to help treat depression.

Funding and Disclosure

This study was funded by the Brazilian National Council for Scientific and Technological Development (CNPq, grants #466760/2014 & #479466/2013), and by the CAPES Foundation within the Ministry of Education (grants #1677/2012 & #1577/2013). The authors declare no competing financial interests.

Acknowledgments

The authors would like to express their gratitude to all patients who volunteered for this experiment. To the Brain Institute and to the Hospital Universitário Onofre Lopes (HUOL), both from the Federal University of Rio Grande do Norte (UFRN) for always giving the necessary institutional support. To the laboratory of Pharmacognosy for helping with placebo preparation. To Edilsom Fernandes, for carefully preparing the Ayahuasca batch used in our study, and for the fruitful discussion, particularly about the dosing session. To Sidarta Ribeiro, for the enthusiastic support throughout the study. To Dr. Ricardo Lagreca, for the unconditional support. To Beatriz Labate, for the fruitful discussions. To Altay Souza and João Sato for assistance with statistical analysis. To Deborah Maia, Ranna Brito, Tayrine Lopes, Lízie Brasileiro, Artur Morais, Isaac Campos, Brígida Albuquerque, Marianna Lucena, Fernanda Araújo, Raíssa Nóbrega, Marina Leonardo, Kaique Andrade, Rodolfo Lira, Giuliana Travassos, for helping with data acquisition. To Prof. Octávio Pontes-Neto and Adriano Tort for critical review of the manuscript. To the Brazilian federal funding agencies CNPq (grants #466760/2014 & #479466/2013) and CAPES (grants #1677/2012 & #1577/2013) for providing financial support.

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Supplementary material
"A randomized placebo-controlled trial on the antidepressant effects of the psychedelic ayahuasca in treatment-resistant depression."

Quantification of ayahuasca Alkaloids

Ayahuasca sample extraction. Ayahuasca alkaloids extraction was performed according to the procedure described in Pires *et al.* (2009). First, 50 µL of sample solution was diluted with deionised water (1:100). Borate buffer 0.25 M pH 9.0 (3 mL) was added into 500 µL of the diluted sample solution and the internal standard diphenhydramine (100 µL of a solution of 10 µg/mL) was loaded onto a C18 cartridge previously conditioned (methanol 2.0 mL, deionised water 1.0 mL and borate buffer 2.0 mL). The loaded cartridge was further washed with deionised water (1.0 mL) and acetonitrile 10% (1.0mL). After drying the cartridges for 7 min, the analytes were eluted with methanol (2.0 mL). Of this solution, 2 µL was injected in the GC-NPD system.

Reagents and chemicals. Hydrogen borate, methanol and acetonitrile were purchased from Merck (Darmstadt, Germany). Classic Sep-Pack® C18 cartridges (360 mg) were purchased from Waters Co. (Bellefonte, PA, USA). N,N-Dimethyltryptamine was obtained from Cerilliant Corporation (Round Rock, Texas, EUA). Diphenhydramine, harmine hydrochloride and harmaline hydrochloride were purchased from Sigma Co. (St Louis, MO, USA). Tetrahydroharmine was synthesized according to the procedure described in Callaway et al. (1996).

GC-NPD analyses. Analyses for N,N-Dimethyltryptamine, harmine, harmaline and tetrahydroharmine were performed using an Agilent gas chromatograph model 6890 equipped with a nitrogen–phosphorous detector and 7683 series automatic injector (Little Falls, DE, USA). Chromatographic separation was achieved on an HP Ultra-2 fused-silica capillary column (25 m × 0.2mm× 0.33 µm film thickness) using ultra-pure- grade nitrogen as carrier gas at 1.0 mL/min in a constant flow rate mode. Injections (2 µL) were made in splitless mode. The injector port and detector temperature was 280°C. The oven temperature was maintained at 70°C for 1 min; programmed at 30°C/min to 120°C, and 20°C/min to 300°C with a hold at 300°C for 4 min. The analytes were identified based on comparison of its relative retention time with the corresponding values of the internal standard diphenhydramine assayed in the same run. Quantification was based upon the ratio of the integrated peak area to the internal standard. The result was multiplied by 100 to compensate for dilution.

Alkaloid concentration. The ayahuasca batch was kept on a refrigerator in amber glass bottles. Two samples of ayahuasca were sent to GC-NPD analyses, at two different moments of the experiment, 11 months apart. Table S1 shows the two separate alkaloid concentration measurements.

Table S1: Alkaloid concentrations measured at two different moments of the experiment.

	May-15	**Apr-16**
DMT (mg/ML)	0.35	0.37
THH (mg/ML)	1.24	1.15
HRL (mg/ML)	0.26	0.21
HRM (mg/ML)	1.75	1.97

Table S2. Individual demographic and clinical characteristics.

	Sex	Age	Education	Employment status	Age at onset, y	Estimated illness duration, y	Number of previous episodes	Current episode, months	Past unsuccessful medications	Past psychotherapy	Past ECT #sessions	Benzodiazepine medications
A1	F	36	Inc. elementary education	Sick leave	26	10	4	3	**SSRI**(2), NDRI, SNRI	Yes	None	CZP
A2	F	54	Inc. elementary education	Unemployed	33	21	3	3	**SSRI**(3), TCA	Yes	None	CZP
A3	F	34	Undergraduate	Employed	28	6	4	2	**SSRI**(2), **NDRI**	Yes	None	CZP
A4	M	52	Inc. elementary education	Unemployed	38	14	2	2	TCA(2), **SSRI**(2)	Yes	None	DZP
A5	F	56	Secondary education	Unemployed	53	3	1	36	**SSRI**(2), **NDRI**	None	None	DZP
A6	F	47	Postgraduate	Employed	35	12	2	5	**SSRI**(2), SNRI	None	None	CZP
A7	M	22	Inc. undergraduate	Studying	17	5	1	60	SNRI(2), NDRI, SSRI, **TCA**	Yes	16	CZP
A8	F	45	Inc. elementary education	Unemployed	29	16	3	12	TCA(2), **SSRI**(2)	Yes	None	CZP
A9	M	19	Secondary education	Studying	18	1	2	4	**SSRI**, TCA	Yes	None	CZP
A10	F	39	Secondary education	Unemployed	38	1	2	6	SSRI(2), **SARI**, TCA, **SNRI**	Yes	None	CZP
A11	F	47	Postgraduate	Unemployed	45	2	3	4	**TCA**, **SSRI**(2)	Yes	None	CZP
A12	F	33	Postgraduate	Employed	21	12	2	12	**SSRI**(3), SNRI(2), **TCA**, MA,	Yes	None	CZP

									NDRI			
A13	F	32	Inc. elementary education	Sick leave	26	6	6	9	TCA(2), SSRI(2)*	Yes	None	BZP
A14	F	40	Elementary education	Unemployed	26	14	3	48	SSRI(2), **SNRI**	None	None	CZP
P1	M	27	Secondary education	Employed	21	6	2	1	**TCA**(2), SSRI(2), NDRI, **SNRI**	Yes	None	AZP
P2	F	50	Secondary education	Unemployed	41	9	4	8	**SSRI**(2), NSSRI	Yes	None	DZP
P3	F	52	Postgraduate	Unemployed	36	16	3	6	**SNRI**, SSRI, TCA	Yes	21	APZ
P4	M	59	Secondary education	Unemployed	57	2	2	8	SSRI(2), NDRI, SARI, **SNRI**	Yes	None	CPZ
P5	F	34	Inc. undergraduate	Unemployed	9	25	8	14	**SSRI**(2), **TCA**(4), SNRI(2), SARI	Yes	None	CPZ
P6	F	49	Inc. elementary education	Unemployed	41	8	3	3	**SSRI**(2), SNRI	None	None	CPZ
P7	F	40	Postgraduate	Employed	38	2	2	6	**SSRI**, TCA	Yes	None	CPZ
P8	F	46	Inc. elementary education	Unemployed	13	33	5	36	**SSRI**(4), SNRI, **TCA**	None	None	CPZ
P9	M	21	Inc. undergraduate	Employed	19	2	1	24	**SSRI**, **NSSRI**	Yes	None	APZ
P10	M	56	Inc. elementary education	Unemployed	17	39	5	9	**SSRI**, **TCA**, SNRI	Yes	None	DZP

P11	F	26	Secondary education	Sick leave	24	2	3	6	**SNRI**, TCA, SSRI	Yes	None	CPZ
P12	F	54	Elementary education	Unemployed	40	14	4	12	**SSRI**(2), TCA	Yes	None	CPZ
P13	M	56	Inc. elementary education	Sick leave	34	20	5	2	**TCA**(2), SSRI	Yes	None	CPZ
P14	F	46	Secondary education	Sick leave	29	17	4	12	**SSRI**(2), SNRI	Yes	None	BZP
P15	F	47	Elementary education	Sick leave	44	2	2	5	**SSRI**(2), **TCA**	None	None	CPZ

A=ayahuasca; P=placebo; F=female; M=male; Inc=incomplete; TCA=tricyclic antidepressant; SSRI=selective serotonin-reuptake inhibitor; NDRI=noradrenaline–dopamine-reuptake inhibitor; NSSRI=noradrenaline and specific serotonin-reuptake inhibitor; SNRI=serotonin–noradrenaline reuptake inhibitor; SARI=serotonin antagonist and reuptake inhibitor; MA=melatonergic antidepressant. ECT = Electroconvulsive Therapy. ALP=alprazolam; BZP=bromazepam; CZP=clonazepam; DZP=diazepam. Current patients' medication(s) before washout period appear in bold. *Patient A13 was not under treatment (for 6 months) at enrolment and did not remember the last medication used.

Table S3: HAM-D average scores and within-group effect sizes (Cohen-d) between baseline and seven days (D7) after dosing.

	HAM-D			
	Baseline		D7	
	Aya	Pla	Aya	Pla
Mean (SD)	24.07 (5.34)	19.73 (4.59)	9.72 (7.39)	16.92 (7.36)
Cohen's d	-	-	2.22	0.46
95% CI	-	-	1.28 to 3.17	-0.27 to 1.18

*Aya=ayahuasca; Pla=placebo; D7=seven days after dosing.

Table S4: MADRS average scores and within-group effect sizes (Cohen-d) between baseline and each endpoints.

	MADRS							
	Baseline		D1		D2		D7	
	Aya	Pla	Aya	Pla	Aya	Pla	Aya	Pla
Mean (SD)	36.14 (6.12)	30.13 (5.55)	12.65 (10.27)	21.49 (10.90)	10.32 (10.44)	19.09 (10.44)	11.58 (10.27)	26.76 (10.11)
Cohen's d	-	-	2.78	1.01	3.05	1.35	2.90	0.41
95% CI	-	-	1.74 to 3.82	0.24 to 1.78	1.94 to 4.16	0.53 to 2.17	1.84 to 3.97	-0.31 to 1.14

*Aya=ayahuasca; Pla=placebo; D1=one days after dosing; D2=two days after dosing; D7=seven days after dosing.

Table S5: Adverse events during the dosing session.

	Ayahuasca	Placebo
Vomiting (n, %)	8 (57%)	0 (0%)
Nausea (n, %)	10 (71%)	4 (26%)
Diarrhea (n, %)	1 (7%)	0 (0%)
Headache (n, %)	6 (42%)	8 (53%)
Anxiety (n, %)	7 (50%)	11 (73%)
Restlessness (n, %)	7 (50%)	3 (20%)

Table S6: Dissociative and psychotomimetic effects. Acute changes were measured after 1h40, 2h40 and 4h ayahuasca or placebo intake. For the Clinician-Administered Dissociative States Scale (CADSS), higher values indicate increased dissociative symptoms. For the Brief Psychiatric Rating Scale Positive subscale - BPRS+, higher values indicate increased psychotomimetic symptoms.

	Ayahuasca				**Placebo**			
	Baseline	1h40	2h40	4h	Baseline	1h40	2h40	4h
CADSS	15.43 ± 17.43	20.80 ± 15.67	18.58 ± 20.10	15.17 ± 17.60	15.40 ± 18.70	14.07 ± 17.20	14.80 ± 20.19	11.20 ± 17.93
BPRS+	1.71 ± 1.98	2.08 ± 1.73	1.31 ± 1.49	0.93 ± 1.27	1.00 ± 1.19	0.53 ± 0.74	0.60 ± 1.12	0.73 ± 1.03

*Values are expressed as mean ± SD.

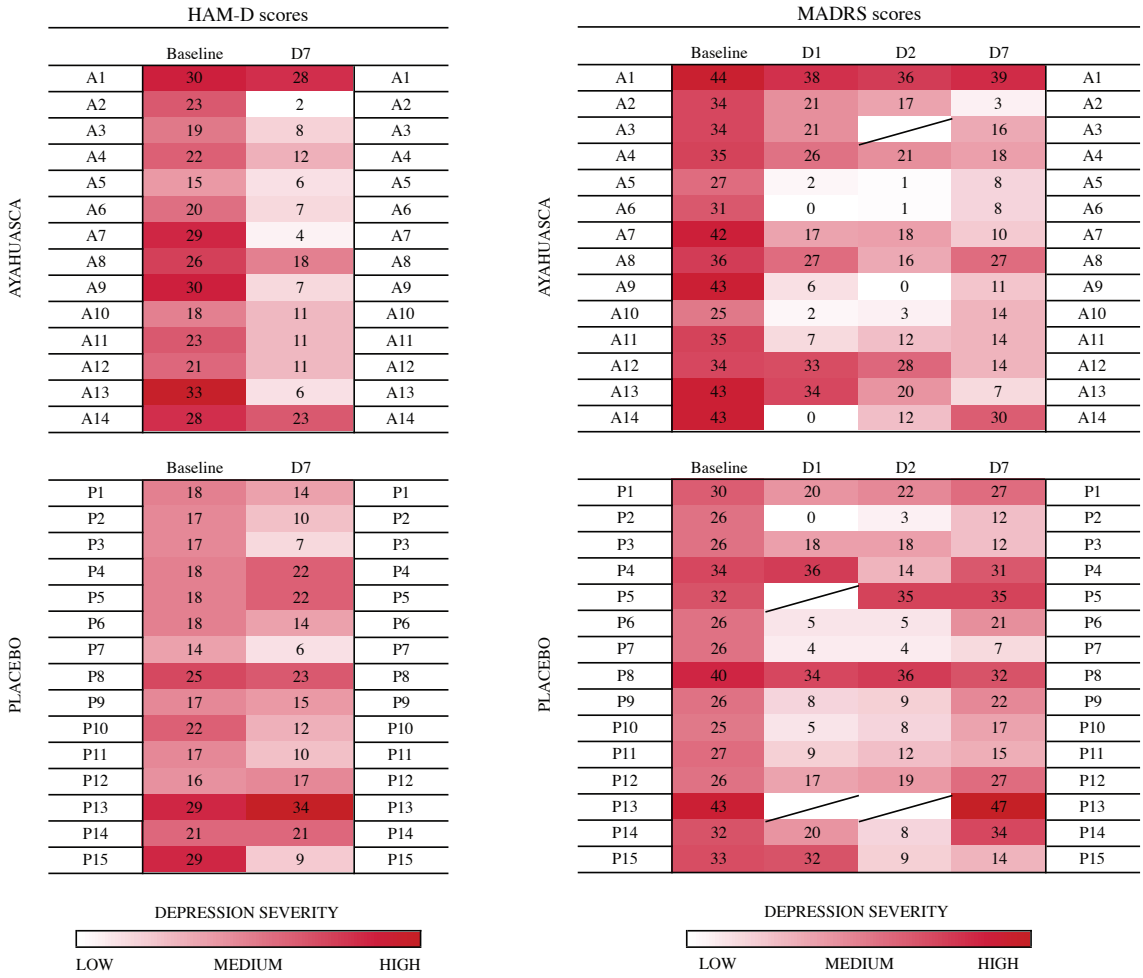

Figure S1. Individual HAM-D & MADRS scores at each endpoint. Reddish colors mean more severe depression, while whitish mean less severity. Note how colors are whitish in the ayahuasca group when compared to the placebo, particularly at D7.

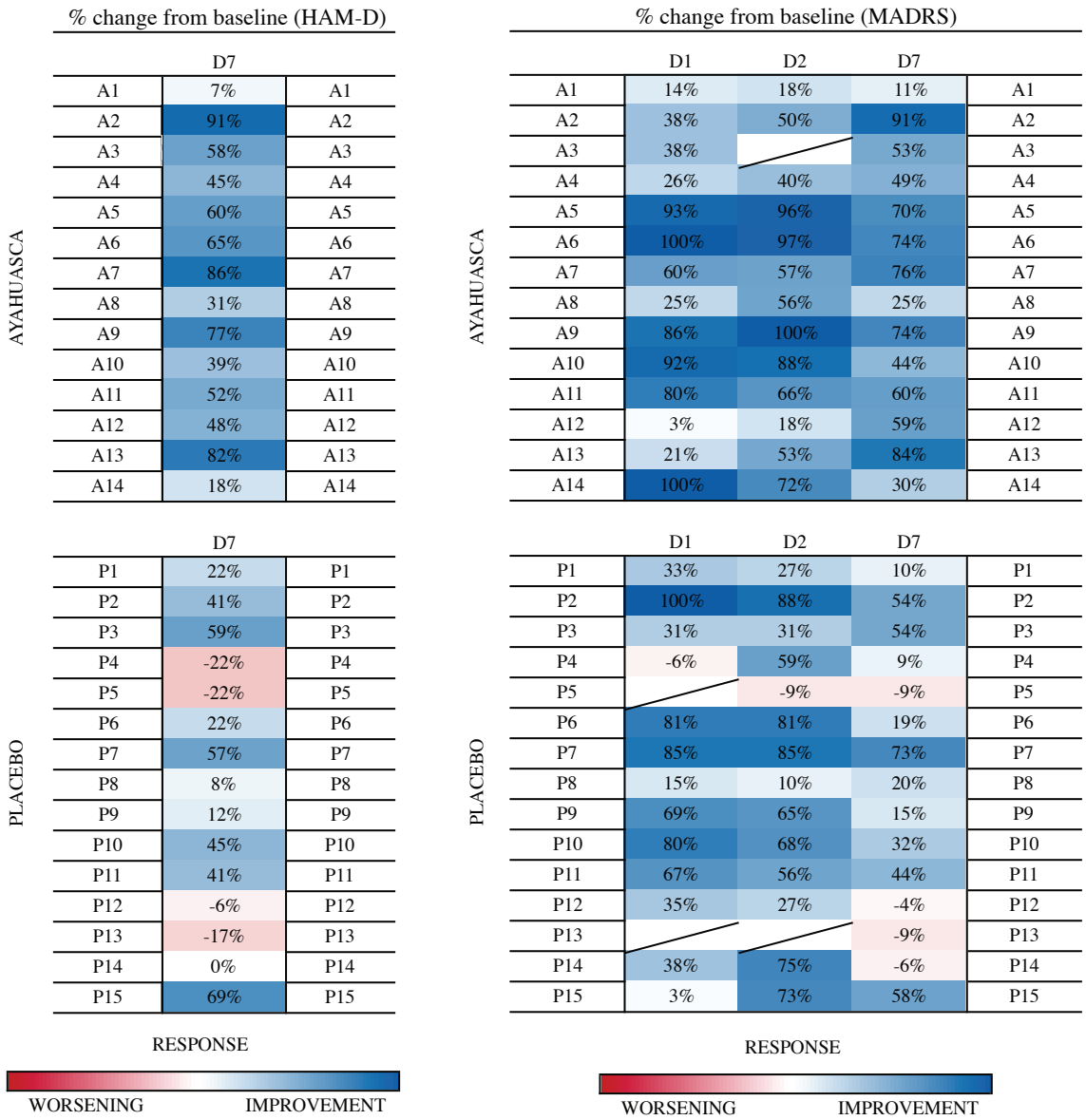

Figure S2. Individual %-change from baseline of MADRS & HAM-D scores at each endpoint. Positive responses appear in bluish, while reddish relates to negative response. All subjects in the ayahuasca group improved, while some patients in the placebo group have worsened slightly: bluish colors (positive response) are predominantly found in the ayahuasca group, when compared to the placebo, that sometimes assumes a reddish pattern (negative response).

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ARTIGO II

Relationships between the antidepressant effects and the psychedelic experience of ayahuasca in treatment-resistant depression

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(Versão inicial do artigo)

Relationships between the antidepressant effects and the acute psychedelic experience induced by ayahuasca in treatment-resistant depression

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Abstract

Ayahuasca is a psychedelic brew used for years as a medicine by indigenous populations from the Amazon basin. Its antidepressant effects have been probed recently in a randomized placebo-controlled trial in patients with treatment resistant depression. We found that a single session with ayahuasca led to a significant rapid decrease in depression symptoms that continued for seven days. During the session, ayahuasca also led to a profound psychedelic experience, which included changes in perception, thoughts, emotion, mood, and mystical-type experiences. In this study we explored the correlations between the antidepressant and the psychedelic effects of ayahuasca. Thirty-five patients with treatment resistant depression volunteered to the study: 18 received ayahuasca, and 17 a placebo substance. We assessed depression severity with the Montgomery–Åsberg Depression Rating Scale (MADRS) applied at baseline, one, two, and seven days after dosing. We assessed the acute psychedelic effects with the Hallucinogen Rating Scale (HRS) and the Mystical Experience Questionnaire (MEQ30). We find evidence that some features of the acute psychedelic experience are related to the antidepressant effect. Most prominently, we observed that changes in perception correlates strongly with the observed decrease in depressive symptoms. Overall, this study suggests that the acute psychedelic experience is related with the antidepressant effects, particularly the reported changes in perception.

Keywords: Psychedelics, Ayahuasca, TRD, Treatment-Resistant Depression, MEQ, MEQ30, Mystical Experience Questionnaire, HRS, Hallucinogen Rating Scale, Peak experience, psychedelic experience.

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Introduction

In recent years we are witnessing a renaissance of the research on the therapeutic value of serotonergic psychedelics, such as ayahuasca, psilocybin and LSD (Moreno et al. 2006; Grob et al. 2011; MacLean et al. 2012; Johnson et al. 2014; Gasser et al. 2015; Osório et al. 2015; Carhart-Harris et al. 2016; Sanches et al. 2016; Griffiths et al. 2016; Ross et al. 2016; Palhano-Fontes et al. 2017). We have recently conducted a double blind placebo-controlled trial in treatment-resistant depression and found that a single session with ayahuasca leads to a significant and rapid antidepressant effect, which was found to be strongest seven days after dosing (Palhano-Fontes et al. 2017).

Ayahuasca is a psychedelic brew used for years as a medicine by indigenous populations from the Amazon basin (Labate and Macrae 2010; Labate and Cavnar 2013). This brew is prepared by decoction of two plants: *Psychotria viridis* that contains the psychedelic N,N-dimethyltryptamine (DMT), a serotonin and sigma-1 receptors agonist (Carbonaro and Gatch 2016), and *Banisteriopsis caapi* that has potent reversible monoamine oxidase A inhibitors, such as harmine, harmaline, and tetrahydroharmine (THH). Ayahuasca may lead to an altered state of consciousness, which includes changes in perception, thoughts, emotion, mood, and often mystical-type experiences, much like a dream state (Riba et al. 2001, 2003; Shanon 2003).

Another peculiarity of psychedelics is the so-called peak experience. Abraham Maslow first introduced this concept in 1964 (R*eligions, Values, and Peak Experiences, 1964*), and it was later brought into the psychedelic field (Maslow 1964). Pahnke referred to the psychedelic peak experience as a mystical-type experience that induces a sense of unity, a transcendence of time and space, a deeply felt positive mood, a sense of sacredness, paradoxicality, a noetic quality, ineffability, and persisting positive changes in different domains, including attitudes and behavior towards the self, others, life and the experience itself (Pahnke 1966, 1969).

It has been suggested that the therapeutic benefit of psychedelics depends on the psychedelic experience, particularly on the peak experience (Bogenschutz et al. 2015; Garcia-Romeu et al. 2015; Majić et al. 2015; Griffiths et al. 2016; Ross et al. 2016). In an open label study of psilocybin for smoking addiction, cessation outcomes were significantly correlated with measures of mystical experience during dosing (Garcia-Romeu et al. 2015). In another study using psilocybin for treating alcohol dependence, mystical experiences were correlated with changes in drinking behavior, decreases in craving and increases in abstinence (Bogenschutz et al. 2015). In addition, recent controlled studies with psilocybin in depression and anxiety in patients with life-threatening cancer showed that mystical-type experience during dosing mediates the therapeutic outcomes (Griffiths et al. 2016; Ross et al. 2016).

Psychedelic effects have been assessed by different psychometric scales, and often includes the Hallucinogen Rating Scale (HRS) (Strassman et al. 1994; Riba et al. 2001, 2003), the Mystical Experience Questionnaire (MEQ30) (Griffiths et al. 2008, 2016; MacLean et al. 2012; Barrett et al. 2015), and the altered state of consciousness ratting scale (Studerus et al. 2010). When applied to studies using freeze-dried ayahuasca, HRS scores peak between 1.5 and 2h after ayahuasca ingestion, and are found to increase significantly in all HRS subscales: intensity, somaesthesia, affect, perception, cognition and volition (Riba et al. 2001, 2003).

In this study, we used data from our randomized placebo-controlled trial in treatment-resistant depression to investigate the correlations between the antidepressant effects of ayahuasca and the acute psychedelic experience. We hypothesized that increased antidepressant effects will be observed in subjects with stronger psychedelic and mystic-type experiences.

Methods

Patients

We recruited 35 adults (18-60 y.o) who met criteria for treatment-resistant unipolar depression as diagnosed by the Structured Clinical Interview for Axis I (DSM- IV). Treatment-resistant depression defines cases of inadequate respond to appropriate courses of at least two antidepressants from different classes (Conway et al. 2017). Selected patients were in a current moderate-to-severe depressive episode at screening (MADRS≥20). We adopted the following ex-

clusion criteria: previous experience with ayahuasca, current medical disease based on history, pregnancy, current or previous history of neurological disorders, history of schizophrenia or bipolar affective disorder, history of mania or hypomania, use of substances of abuse, and imminent suicidal risk. All trial procedures took place at the Onofre Lopes University Hospital (HUOL), Natal-RN, Brazil. The University Hospital Research Ethics Committee approved the study (# 579.479), and all volunteers provided written informed consent before participation.

Instruments

We assessed depression severity with the Montgomery–Åsberg Depression Rating Scale (MADRS) (Montgomery and Asberg 2006). MADRS was applied at baseline, one day, two days and seven days after dosing. This scale is composed of 10 items and should be applied by a trained psychiatrist.

The Hallucinogen Rating Scale (HRS) was originally developed to evaluate the psychedelic effects induced by DMT (Strassman et al. 1994). It is divided into six subscales: **intensity**, which reflects the strength of the overall experience; **somaesthesia**, which assesses somatic effects such as interoception, visceral and tactile effects; **affect**, assesses comfort, emotional and affective effects; **perception**, assesses visual, auditory, gustatory, and olfactory effects; **cognition**, assesses changes in thought processes and content; and, **volition**, which assesses the capacity to willfully interact with his/her "self" and/or the environment. Herein, we used the HRS version translated to Brazilian Portuguese (Mizumoto et al. 2011). Patients completed the HRS scale at the end of the dosing session, when the acute effects had already ceased.

The Mystical Experience Questionnaire (MEQ30) was designed to assess experiences of mystical-type (MacLean et al. 2012; Barrett et al. 2015). The questionnaire has four empirically-derived factors: **mystical**, comprising items about unity, noetic quality, and sacredness; **positive mood**, assesses mood and emotional effects; **transcendence of time and space**, assesses changes in perception of time and space; and **ineffability**, which assesses the difficulty in describing the experience using words (MacLean et al. 2012). Patients completed the MEQ30 at the end of the dosing session, when the acute effects had already ceased.

Study design and procedures

After a washout period of ~2-week, patients had a baseline psychiatric evaluation, when the MADRS baseline assessment was completed. Dosing session lasted for about 8 hours. Half of the patients received a placebo substance with emetic properties, and the other half a single dose of 1 ml/kg of ayahuasca adjusted to contain 0.36 mg/kg of DMT. During dosing sessions, patients remained in a silent and comfortable room, with a bed, a recliner, and controlled temperature, natural and dimmed light. Patients were allowed to listen to a predefined music playlist through headphones. They were oriented to stay quiet, with their eyes closed, and to focus on their body, thoughts and emotions. At least two investigators remained next to the patients throughout the session. At the end of the session, patients were asked to describe their experience, and to respond to the HRS and MEQ30 questionnaires. Patients returned to the hospital for three follow-up psychiatric assessments, when the MADRS was applied: one day (D1), two days (D2), and seven days (D7) after dosing. The full protocol is described elsewhere (Palhano-Fontes et al. 2017).

Statistical analysis

We used the Mann-Whitney test to assess differences in the psychedelic experience (HRS and MEQ30 scores) between the ayahuasca and the placebo groups. Significance was set at $p < 0.05$, two-tailed. The correspondence between the acute psychedelic effects and depression severity was computed using Pearson correlation. Correlations were evaluated between the following measures: i) HRS scores and MADRS score change at D7 in each group separately; ii) HRS scores and MADRS scores changes at D7 from responders only; iii) HRS scores and MADRS scores changes at D7 from ayahuasca responders only; iv) MEQ30 scores and MADRS score changes at D7 in each group separately;

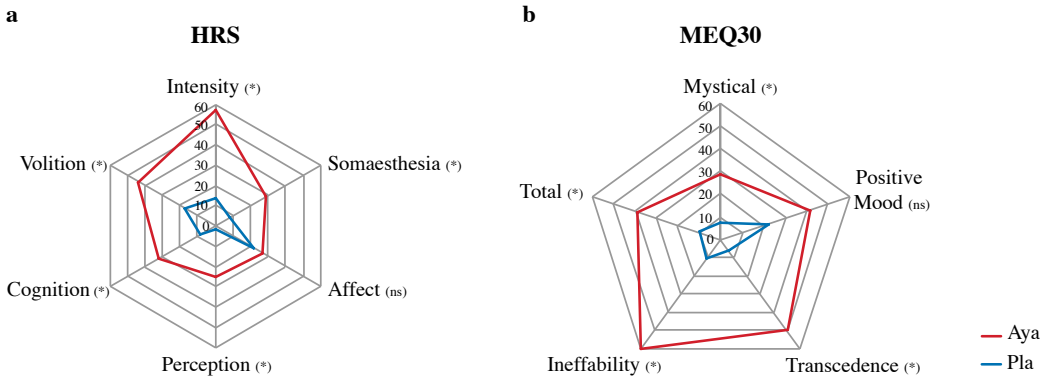

Figure 1 – HRS subscales and MEQ factors scores assessing the psychedelic experience during the dosing session. The ayahuasca group is presented in red, and the placebo in blue. **a)** We observed significantly higher scores in the ayahuasca group in five HRS subscales: perception ($p<0.0001$), somaesthesia ($p< 0.0001$), cognition ($p<0.0001$), intensity ($p<0.0001$), and volition ($p=0.0003$). Only affect was not different between ayahuasca and placebo ($p = 0.38$). **b)** We observed significantly higher MEQ30 scores in the ayahuasca group in three factors: mystical ($p=0.049$), transcendence of time and space ($p=0.0008$) and ineffability ($p=0.003$), except for the positive mood ($p=0.32$). Total MEQ30 score is also significantly different ($p=0.004$). Values are expressed as a percentage of maximum possible score.

v) MEQ30 scores and MADRS score changes at D7 from responders only; vi) MEQ30 scores and MADRS score changes at D7 from ayahuasca responders only. Response was defined as a reduction of at least 50% from MADRS baseline scores (Conway et al. 2017). Multiple comparisons correction was based on the number of factors of each scale (corrected p-value): N= 6 ($p<0.0083$) for the HRS and N=5, ($p<0.01$) for the MEQ30.

Results

Out of the 35 patients, 27 responded the HRS: 13 in the ayahuasca group, 14 in the placebo group. The MEQ30 was added to the study protocol with the ongoing trial. Therefore, only 15 patients responded the MEQ30: 8 in the ayahuasca group, 7 in the placebo.

Figure 1 shows the HRS and MEQ30 scores for both groups (ayahuasca and placebo), assessing the psychedelic experience during the dosing session. Both scales are very sensitive to the psychedelic effects, and we find significant higher scores in the ayahuasca group than in the placebo. Figure 1a shows the HRS results for all six subscales. We find significant differences between groups in five of them: perception ($p<0.0001$), somaesthesia ($p<0.0001$), cognition ($p<0.0001$), intensity ($p<0.0001$), and volition ($p=0.0003$). Only affect was not significantly different between groups ($p=0.38$). When correcting for multiple comparison, the results in all five subscales are still significant. Figure 1b shows the MEQ30 scores for both groups. We find significant between-groups differences in mystical ($p=0.049$), transcendence of time and space ($p=0.0008$), ineffability ($p=0.003$), and in the total MEQ30 score ($p=0.004$). Only the positive mood r was not significantly different between groups ($p=0.32$). When correcting for multiple comparisons, the mystical factor is no longer significant, but the other factors remain. Average and standard error of mean (SEM) for each factor (HRS and MEQ) are described in supplementary table S1. Supplementary figures S1 and S2 show average, standard error and individual values for HRS subscales and MEQ30 factors, respectively.

Correlations between HRS subscale scores and changes in MADRS scores from baseline to D7 were not statistically significant when assessing each group separately (ayahuasca and placebo). Results are detailed in figure S3.

Figure 2 shows the significant correlations found when analyzing the subgroup of patients who responded to the treatment seven days after dosing from both groups. We find a positive significant correlation with three HRS subscales: perception ($r=0.92$, $p<0.0001$), cognition ($r=0.73$, $p=0.007$) and somaesthesia ($r=0.69$, $p=0.012$). The remain subscales (volition, intensity and affect) was not significantly correlated with

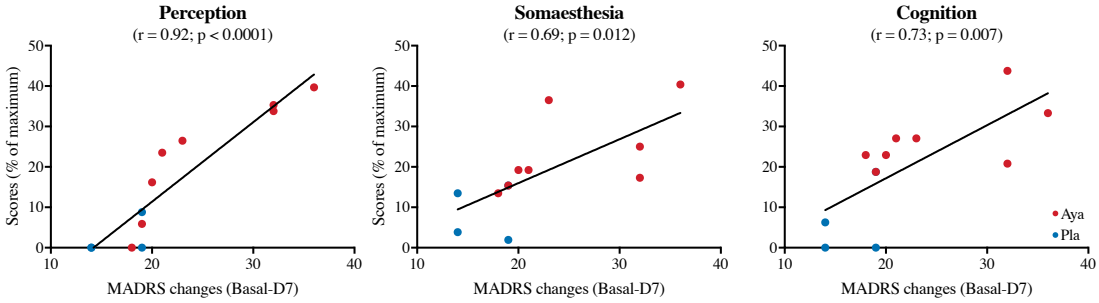

Figure 2 – Pearson's correlations between HRS subscales and MADRS score changes at D7 including responders only. We observe significant positive correlations with three HRS subscales: perception (r=0.92, p<0.0001), somaesthesia (r=0.69, p=0.012), and cognition (r=0.73, p=0.007).

MADRS scores changes at D7 (Figure S4). Correlations with perception and cognition remain significant after multiple comparisons correction.

Figure 3 shows the correlations between HRS subscales and MADRS changes at D7, when including responders from the ayahuasca group only. We observe a positive significant correlation with only perception (r=0.90, p=0.002) subscale, which survives multiple comparisons correction. Other HRS subscales were not significantly correlated with MADRS changes at D7 (Figure S5).

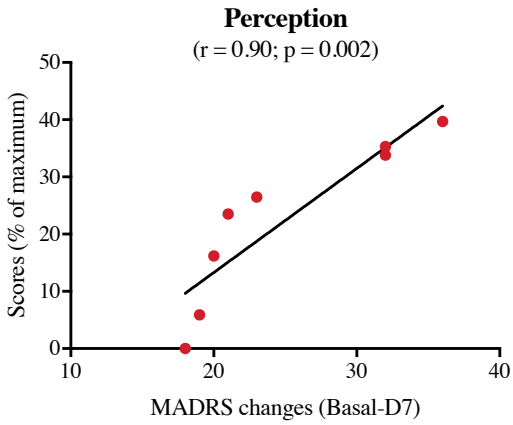

Figure 3 – Pearson's correlation between HRS subscales and MADRS score changes at D7 including ayahuasca responders only. We observe a significant positive correlation between perception (r=0.90, p=0.002) and changes in MADRS at D7.

Despite the small number of patients who completed the MEQ scale, we find a negative correlation between changes in MADRS scores and transcendence of time and space in patients in the ayahuasca group (r=-0.84, p=0.009), which survives multiple comparison correction (Figure 4). The remaining three factors (ineffability, mystical and positive mood) and MEQ30 total score were not significantly correlated with MADRS score changes (Figure S6). We did not find significant correlations when considering responders, or ayahuasca responders only (Figure S7 and S8, respectively).

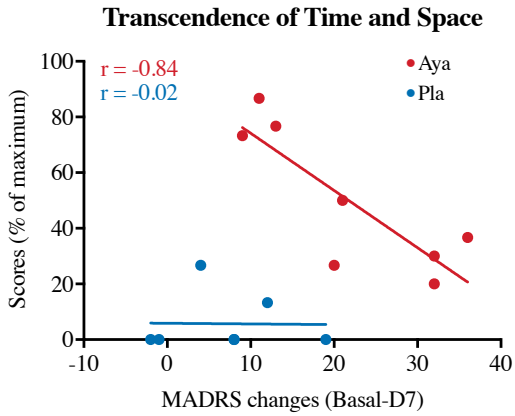

Figure 4 – Pearson's correlation between transcendence of time (MEQ30) and MADRS score changes at D7 in the each group separately. We observe significant negative correlation with transcendence of time and space (r=-0.84, p=0.009) in the ayahuasca group.

Discussion

In this study, we explored the relationship between the acute psychedelic experience of a single dose of ayahuasca and its antidepressant effects in patients with treatment-resistant depression. Data come from our recently conducted randomized placebo-controlled trial, in which we observed a rapid antidepressant effects of a single dose of ayahuasca, starting one day after dosing and persisting for seven days (Palhano-Fontes et al. 2017). We found significant changes in most MEQ30 and HRS subscales during the acute psychedelic effects. Scores in both scales were significantly higher in the ayahuasca group than in the placebo. Furthermore, decreased MADRS scores from baseline to D7 were positively correlated

with HRS subscales, particularly perception. Positive antidepressant effects were also inversely correlated with transcendence of time and space, from the MEQ30 scale.

Recent evidence supports the observed antidepressant effects of ayahuasca. First, the presence of N,N-DMT, which acts primarily as a serotonergic and sigma-1 agonist (Smith et al. 1998; Fontanilla et al. 2009; Carbonaro and Gatch 2016; Nichols 2016), both been implicated in the pathophysiology of depression (Cai et al. 2015; Otte et al. 2016). Second, the presence of MAOi in ayahuasca directly affects monoaminergic systems, increasing the concentration of monoamines such as serotonin, noradrenaline and dopamine, and all of these appear to be modified in depression (Cai et al. 2015; Otte et al. 2016). Third, THH functions as a serotonin reuptake inhibitor (Riba et al. 2003), which forms the basis of many antidepressants, such as selective serotonin reuptake inhibitors (SSRI).

Besides broad increases in the concentration of certain neurotransmitters, we should also take into account subtler mechanisms related to psychological aspects of the psychedelic experience. In fact, psychotherapy, such as cognitive-behavioral therapy, is an effective tool for treating depression (DeRubeis et al. 2005; Hofmann et al. 2012). In fact, prior studies have suggested that the mystical experience induced by psilocybin is associated with reduction in symptoms of depression and anxiety in patients with life-threatening cancer (Griffiths et al. 2016; Ross et al. 2016). Furthermore, a trial using psilocybin in the treatment of tobacco addition observed that smoking cessation outcomes were significantly correlated with measures of mystical experience during dosing (Garcia-Romeu et al. 2015). These results are further corroborated by studies in healthy individuals submitted to a moderate-to-high dose of psilocybin showing long-term positive changes in mood, behavior and personality (Griffiths et al. 2008, 2011).

Previous use of the MEQ30 to evaluate the acute effects of ayahuasca is restricted to a study that reanalyzed the factorial MEQ30 structure for individuals with experience with ayahuasca (Bouso et al. 2016). We found a significantly increased MEQ30 scores in the ayahuasca group, when compared to placebo, in both the total MEQ30 scores, as well as in its factors: mystical, transcendence of time space, and ineffability. Positive mood was not statistically significant, as the placebo group also scored high in this factor. Correlation between MADRS score changes at D7 was limited to an inverse relationship with transcendence of time and space. Besides the small number of patients that completed the MEQ30 scale (N=15, 8 in ayahuasca group), it should be noted that all of our patients were naïve to any psychedelic substances, and the experience with ayahuasca was the first contact they had with an altered state of consciousness of this type. Thus, distinguishing different aspects of the experience was a difficult task. Changes in perception, as visions, or in body sensation, could be easily merged with more subjective and subtle effects, as the sense of unity with the universe or the transcendence of time and space. Furthermore, most of our patients received low formal education, which hampers the understanding of some words and concepts used in the MEQ30, such as "ultimate reality", "spiritual height", "timelessness", "ecstasy", "tenderness", etc.

The HRS scores were significantly higher for the ayahuasca group than the placebo in five out of the six HRS subscales: intensity, somaesthesia, perception, cognition and volition. When compared to a previous study with healthy volunteers, while we did not find differences in the affect subscale, their study found that only volition was not statistically significant (Riba et al. 2003).

The scores we find for both scales (MEQ30 and HRS) can be considered moderate, when compared to controlled studies with psilocybin and ayahuasca (Riba et al. 2001, 2003; Griffiths et al. 2016). In fact, the dose of N,N-DMT we used (0.36 mg/Kg) in our study is low. Higher N,N-DMT concentrations of 0.75 or 1 mg/Kg were associated with higher HRS scores reported previously in studies with ayahuasca in healthy subjects (Riba et al. 2001; Dos Santos et al. 2012).

We find significant positive correlations between the observed improvement of depression symptoms (MADRS scores) and three HRS subscales in patients who responded to the treatment: perception, cognition and somaesthesia. Despite the small numbers of subjects (N = 8), we also examined what happened when including

only ayahuasca responders. Again, a positive correlation was found, but now restricted to perception subscale.

Although it seems that several HRS dimensions are important to the clinical outcome, changes in the perception should be highlighted. It encompasses auditory and vision changes, and showed the greatest correlation coefficients in all the analyses performed. Ayahuasca is well known for the visions it elicits, to a point that some ayahuasca traditions give a special name for this phenomenon, and call it *mirações* (seeings). The content of the visions varies significantly, and can span from kaleidoscope images, animals, to complex conversations with a stranger (Shanon 2003). A previous fMRI study with volunteers under the acute effects of ayahuasca has shown that the activity of the primary visual cortex when seeing pictures with the eyes open is equivalent to that found during a mental imagery task with the eyes closed (De Araujo et al. 2012). In fact, it has been suggested that visions may play an important role in the therapeutic effect of ayahuasca, as they could help to bring reflections on personal relationships, traumatic events and provide personal insights (Frecska et al. 2016), and our results give support to this hypothesis.

Previous studies found that the mystical-type experience has an specific role apart from overall intensity of the psychedelic effects measured by the HRS (Griffiths et al. 2016; Ross et al. 2016). On the other hand, a recent study of psilocybin-assisted treatment for alcohol dependence found that both total MEQ30 score and HRS intensity are significantly correlated with positive changes in drinking behavior (Bogenschutz et al. 2015). In our study, we find that perception is strongly correlated with clinical outcome, suggesting that other dimensions, apart from the mystical experience, may play a role in the therapeutic response of psychedelics.

Overall, our study suggests that the more intense the psychedelic effects measured by HRS the more robust were the antidepressant effects observed. Particularly, our data support that alterations in perception during the psychedelic experience may account for the psychological processes involved in the therapeutic value of psychedelic compounds.

Funding and Disclosure

This study was funded by the Brazilian National Council for Scientific and Technological Development (CNPq, grants #466760/2014 & #479466/2013), and by the CAPES Foundation within the Ministry of Education (grants #1677/2012 & #1577/2013). The authors declare no competing financial interests.

Acknowledgements

We are thankful to all patients who volunteered to the study. We are also thankful to the Brain Institute and to the Hospital Universitário Onofre Lopes (HUOL), of the Federal University of Rio Grande do Norte (UFRN), for giving the necessary institutional support. To Edilsom Fernandes, for carefully preparing the ayahuasca batch used in our study, and for the fruitful discussion, particularly about the dosing session. To Dayanna Barreto, Deborah Maia, Ranna Brito, Tayrine Lopes, Lízie Brasileiro, Morgana Novaes, and Jéssica Pessoa, and Kátia Andrade, for helping with data acquisition. To Diego Laplagne for critical review of the manuscript.

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Supplementary Material

Table S1. Average HRS and MEQ ratings for each subscale and factor assessing the psychedelic experience during the dosing session.

	Ayahuasca	**Placebo**	
Hallucinogen Rating Scale			
Perception	25.11 (4.22)	1.79 (1.00)	$p < 0.0001$ (****)
Somaesthesia	28.99 (3.74)	8.52 (2.02)	$p < 0.0001$ (****)
Cognition	32.05 (2.63)	8.48 (2.19)	$p < 0.0001$ (****)
Intensity	57.47 (6.39)	13.45 (3.40)	$p < 0.0001$ (****)
Volition	43.51 (5.06)	17.63 (2.92)	$p = 0.0003$ (***)
Affect	26.47 (3.52)	22.06 (3.42)	$p = 0.38$ (ns)
Mystical Experience Questionnaire			
Mystical	28.67 (8.04)	7.048 (2.17)	$p = 0.0493$ (*)
Positive Mood	41.67 (11.95)	22.38 (8.78)	$p = 0.32$ (ns)
Transcendence of time and space	50 (9.08)	5.71 (3.96)	$p = 0.0008$ (***)
Ineffability	60 (11.13)	10.48 (6.80)	$p = 0.0031$ (**)
Total	38.67 (5.81)	10.19 (2.32)	$p = 0.0037$ (**)

All data are expressed as a percentage of maximum possible score. Data are means (Standard Error of Mean). Asterisks indicate significant differences between ayahuasca and placebo (*$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$, p-value uncorrected).

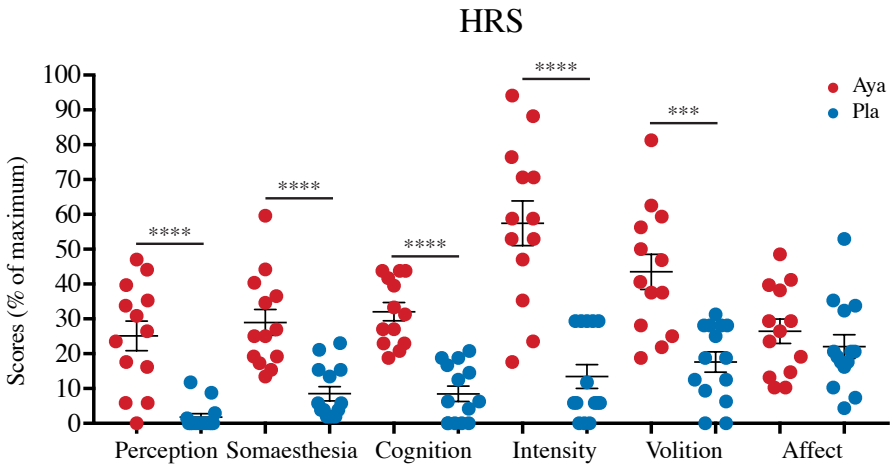

Figure S1. Individual scores for each HRS subscale. The ayahuasca group is presented in red, and the placebo in blue. Dots represent individual scores. Bars represent mean ± SEM. We found significant differences between groups in five subscales: perception ($p<0.0001$), somaesthesia ($p<0.0001$), cognition ($p<0.0001$), intensity ($p<0.0001$), and volition ($p=0.0003$). Only affect was not significantly different between groups ($p=0.38$). Asterisks indicate significant differences between ayahuasca and placebo (*$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$, p-value uncorrected).

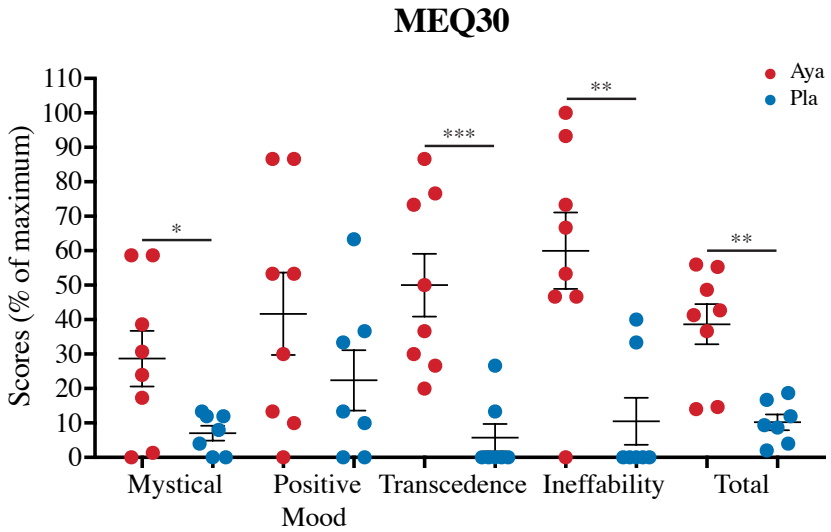

Figure S2. Individual MEQ30 scores for each factor. The ayahuasca group is presented in red, and the placebo in blue. Dots represent each individual score. Bars represent mean ± SEM. We found significant differences between groups in mystical (p=0.049), transcendence of time and space (p=0.0008) and ineffability (p=0.003) factors, and in the total MEQ30 scores (p=0.004). Only the positive mood factor (p=0.32) was not significantly different between groups. Asterisks indicate significant differences between ayahuasca and placebo (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, p-value uncorrected).

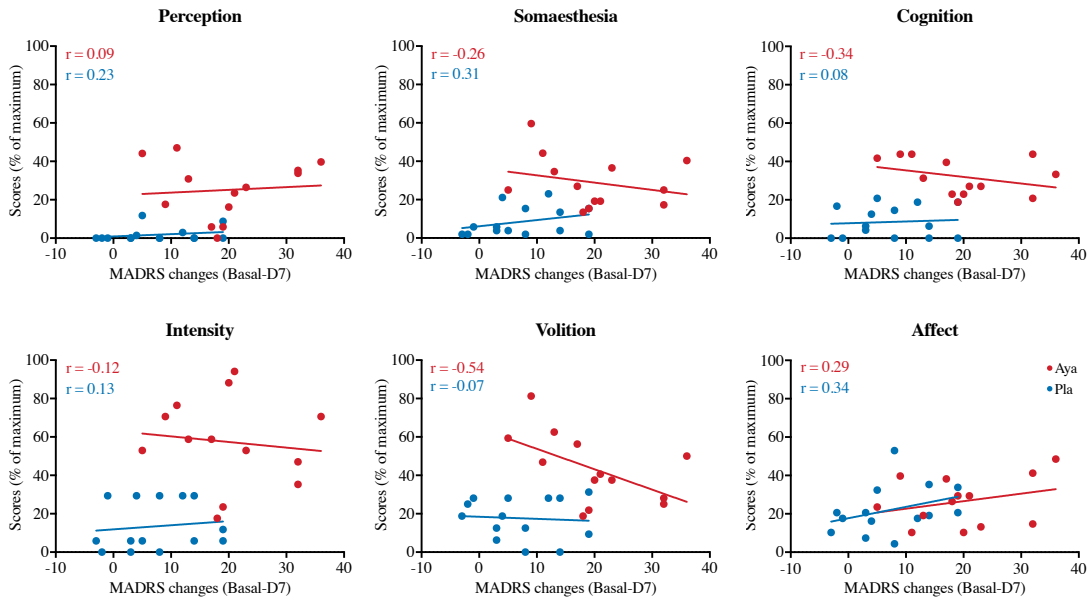

Figure S3. Correlations between MADRS score changes from baseline to D7 with HRS subscales, in each group separately. No significant correlations were found. For ayahuasca group: perception (r=0.09, p=0.78), somaesthesia (r=-0.26, p=0.39), cognition (r=-0.34, p=0.26), intensity (r=-0.12, p=0.70), volition (r=-0.54, p=0.06) and affect (r=0.29, p=0.34). For placebo group: perception (r=0.23, p=0.42), somaesthesia (r=0.31, p=0.27), cognition (r=0.08, p=0.78), intensity (r=0.13, p=0.67), volition (r=-0.07, p=0.81) and affect (r=0.34, p=0.23).

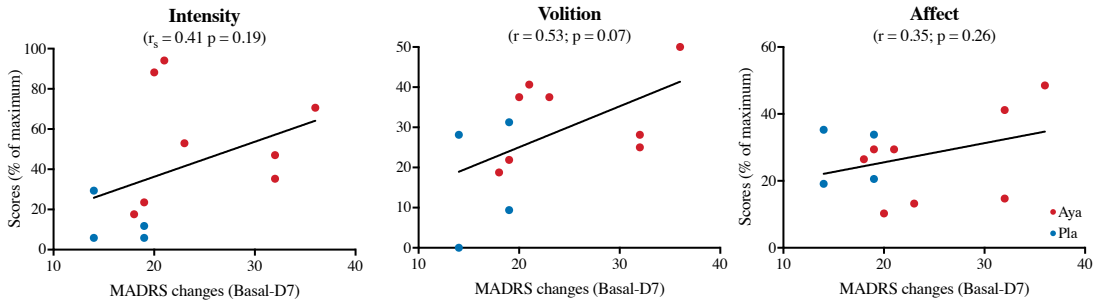

Figure S4. Correlations between MADRS scores changes with the HRS subscales in the group of responders only. Correlations between MADRS score changes from baseline to D7 with intensity (r=0.41, p=0.19), volition (r=0.53, p=0.07) and affect (r=0.35, p=0.26) were not statistically significant.

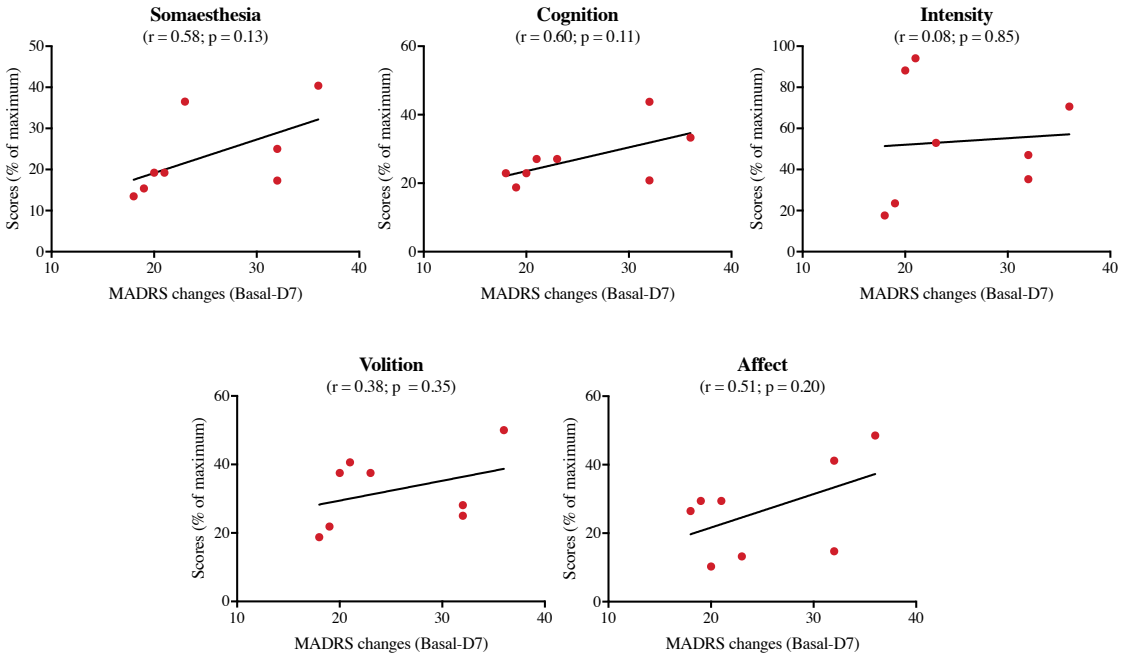

Figure S5. Correlations between MADRS score changes and the HRS in ayahuasca responders only. Correlations between MADRS score changes from baseline to D7 with the somaesthesia (r=0.58, p=0.13), cognition (r=0.60, p=0.11), intensity (r=0.08, p=0.85), volition (r=0.38, p=0.35), and affect (r=0.51, p=0.20) subscales were not significant when considering ayahuasca responders only.

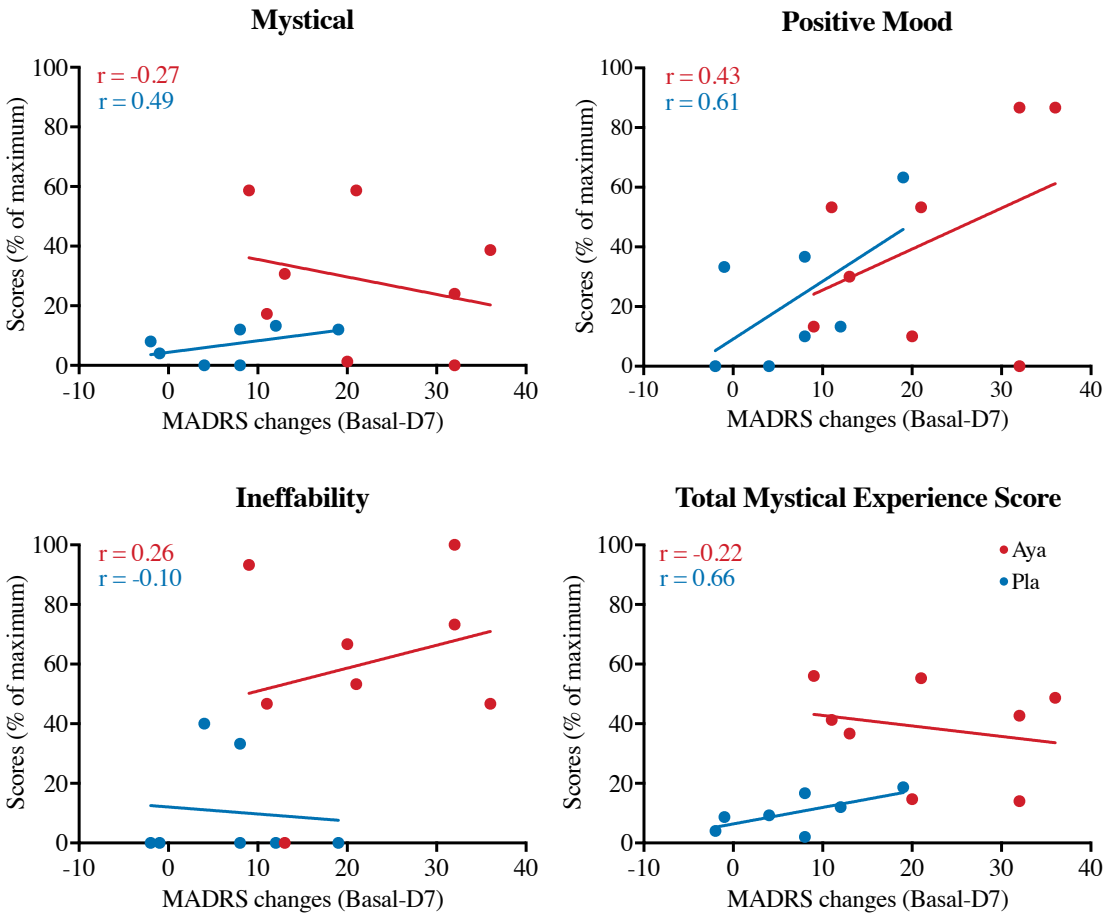

Figure S6. Correlations between MADRS score changes and MEQ30 scores in each group separately. **Following** correlations were not statistically significant for ayahuasca group: mystical (r=-0.27, p=0.52), positive mood (r=0.43, p=0.29), ineffability (r=0.26, p=0.54) and MEQ30 total score (r=-0.22, p=0.59); and for placebo group: mystical (r=0.49, p=0.26), positive mood (r=0.61, p=0.14), ineffability (r=-0.10, p=0.84) and MEQ30 total score (r=0.66, p=0.10).

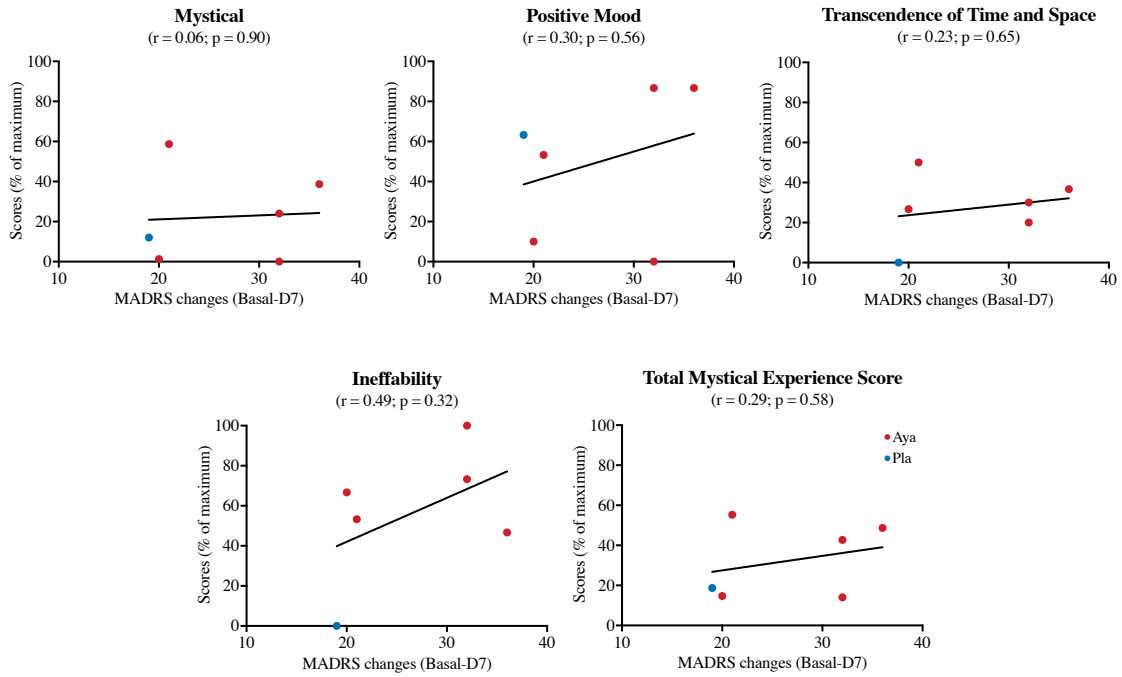

Figure S7. Correlations between MADRS scores changes with MEQ30 in responders only. Correlations between MADRS score changes from baseline to D7 with mystical (r=0.06, p=0.90), positive mood (r=0.30, p=0.56), transcendence of time and space (r=0.23, p=0.65), ineffability (r=0.49, p=0.32) and MEQ30 total score (r=0.29, p=0.58) were not statistically significant when only responders, from both groups, were considered.

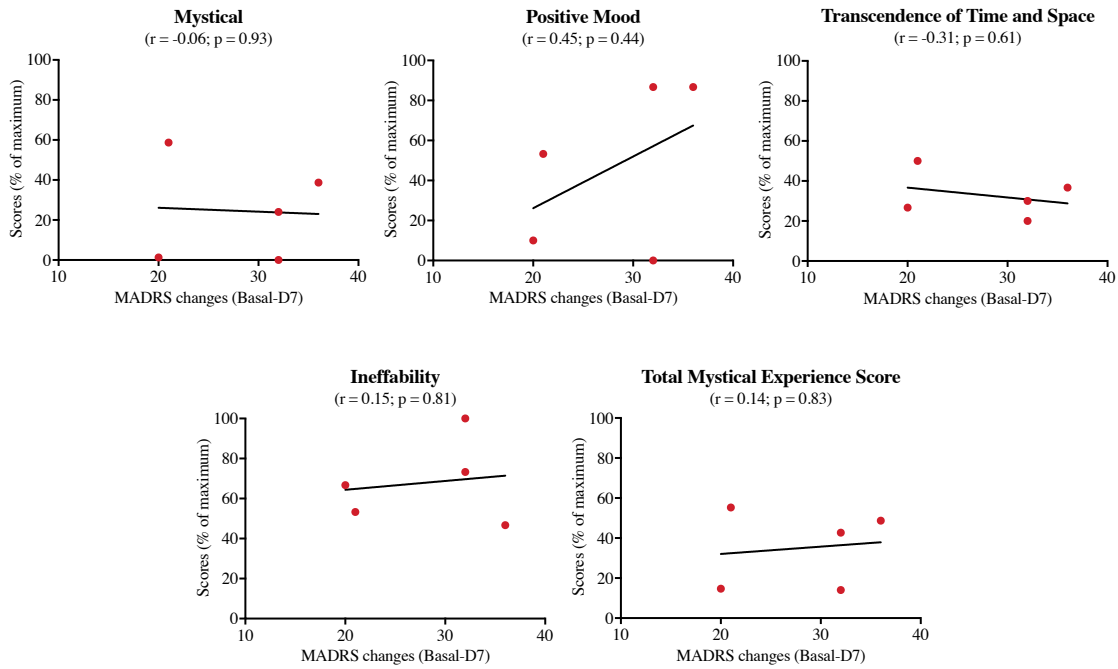

Figure S8. Correlations between MADRS scores changes with MEQ30 in ayahuasca responders only. Correlations between MADRS score changes from baseline to D7 with mystical (r=-0.06, p=0.93), positive mood (r=0.45, p=0.44), transcendence of time and space (r=-0.31, p=0.61), ineffability (r=0.15, p=0.81) and MEQ30 total score (r=0.14, p=0.83) were not statistically significant when only responders, from both groups, were considered.

ARTIGO III

Emotion processing and the antidepressant effects of ayahuasca in treatment-resistant depression: an fMRI study

Fernanda Palhano-Fontes, Heloisa Onias, Morgana Novaes, Kátia C. Andrade, Emerson Arcoverde, João P Maia-de-Oliveira, Dráulio B. de Araújo

(Versão inicial do artigo)

Emotion processing and the antidepressant effects of ayahuasca in treatment-resistant depression: an fMRI study

Fernanda Palhano-Fontes,[1,2] Heloisa Onias,[1,2] Morgana Novaes,[1,2] Katia C Andrade,[1,2] Emerson Arcoverde,[2] Joao P Maia-de-Oliveira,[2] Draulio B Araujo[1,21*]

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Abstract

Major depression is a disorder intrinsically related to emotion. Neuroimaging studies in patients with depression find structural and functional changes in brain circuits involved in emotional processing, such as the amygdala, the cingulate cortex, insula and a number of regions in the prefrontal cortex. These same brain regions have been associated with the acute effects of serotonergic psychedelics, such as ayahuasca. Furthermore, in a recent randomized placebo-controlled trial we find that a single session with ayahuasca leads to a significant antidepressant effect, already one day after the session with ayahuasca, compared to placebo. Herein, we explore the subacute effect of ayahuasca in brain circuits involved in emotion processing of patients with depression. Twenty-four patients with treatment resistant depression completed two fMRI sessions: one day before and one day after a dosing session with ayahuasca or placebo. In both sessions they performed a self-emotional regulation task. Depressive symptoms were assessed using the Montgomery–Åsberg Depression Rating Scale (MADRS) at baseline, one, two and seven days after dosing. fMRI results show a significant treatment-by-time interaction in the subgenual anterior cingulate cortex, amygdala, anterior insula, and ventrolateral prefrontal cortex. Data also suggest that changes in the activity of the subgenual cortex are correlated with the observed decreased symptoms of depression one day after dosing. Overall, this study shows that a single ayahuasca session is related with subacute changes in regions involved in the emotional processing, and these changes, particularly in the subgenual cortex, are correlated with the clinical outcome in patients with treatment-resistant depression.

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Introduction

Depression is a disorder intrinsically related to emotion. Studies in patients with major depression disorder have found structural and functional abnormalities in brain circuits implicated in emotional processing (Drevets 2000; Sacher et al. 2012; Sheline 2003). Depression also impairs emotion regulation, defined as the controlled or automatic regulation of the emotional impact of a stimulus (Ochsner and Gross 2005). Results have shown dysfunctional emotional processing in the amygdala, ventral striatum, anterior cingulate cortex, subgenual anterior cingulate cortex, medial orbitofrontal cortex, and parahippocampal areas of patients with depression (Groenewold et al. 2013).

Within the anterior cingulate cortex (ACC), the subgenual portion (sgACC) appears to be involved with depression. Most consistently, studies find increased metabolic activity of this area in patients with depression when compared to healthy controls (Drevets et al. 2008; Mayberg et al. 1997). Decreasing its activity, on the other hand, is frequently observed after therapy for instance with antidepressant medication, psychotherapy, electroconvulsive therapy, deep brain stimulation, and repetitive transcranial magnetic stimulation (Delaveau et al. 2011; Siegle et al. 2012; Mayberg et al. 2005; Mottaghy et al. 2002; Nobler et al. 2001). Similarly, the amygdala is hyperactive in depression, and antidepressant interventions lead to its regulation (Sheline et al. 2001; Godlewska et al. 2012; Williams et al. 2015; Fu et al. 2004).

In parallel, recent studies suggest that serotonergic psychedelics, such as ayahuasca, LSD, and psilocybin, also modulate the activity of brain circuits related to emotional processing, such as the amygdala, insula, prefrontal cortex, and the default mode network (DMN) (Mueller et al. 2017; Carhart-Harris et al. 2012; Palhano-Fontes et al. 2015; Riba et al. 2006; Kraehenmann et al. 2015). Furthermore, clinical trials with psychedelics are starting to test its antidepressant effects, such as ayahuasca and psilocybin (Ross et al. 2016; Griffiths et al. 2016; Sanches et al. 2016; Osório et al. 2015; Palhano-Fontes et al. 2017).

We have recently conducted one open label trial and one randomized placebo-controlled trial with ayahuasca for treatment resistant depression (Osório et al. 2015; Sanches et al. 2016; Palhano-Fontes et al. 2017). Ayahuasca is a decoction used for years as a medicine by Amerindians. It contains the psychedelic tryptamine N,N-Dimethyltryptamine (N,N-DMT), and monoamine oxidase inhibitors (Riba et al. 2001; Riba et al. 2003; Callaway et al. 1999). In the open-label trial, 17 patients participated in a single session with ayahuasca. The results show a rapid and persisting reduction of depressive symptoms, already one day after the session, persisting for 21 days (Osório et al. 2015; Sanches et al. 2016). Similar results were observed in the placebo-controlled trial, and a significant antidepressant effect was observed one day after the session, lasting for seven days, this time compared to placebo (Palhano-Fontes et al. 2017). Patients from this trial were also enrolled in two functional magnetic resonance imaging acquisitions (fMRI), one day before and one day after the session with ayahuasca or placebo.

In this study we explored post-acute changes in brain activity during the performance of an emotional regulation task, and furthermore, the relationship between the fMRI changes and the antidepressant effects of ayahuasca.

Materials and methods

Patients

Twenty-four patients aged 18-60 years who met criteria for unipolar major depressive disorder as diagnosed by the Structured Clinical Interview for Axis I (DSM- IV) participated in this study. Only treatment-resistant depression patients were selected, defined herein as those with inadequate responses to at least two antidepressant medications from different classes (Conway et al. 2017). Selected patients were in a current depressive episode of moderate-to-severe at screening (MADRS≥20), with the following exclusion criteria: previous experience with ayahuasca; current medical disease based on history, physical examination and routine hematologic and biochemical tests; pregnancy; current or previous history of neurological disorders, psychosis, schizophrenia or bipolar affective disorder; history of mania or hypomania; use of substances of abuse; and suicidal risk.

All procedures took place at the Onofre Lopes University Hospital (HUOL), Natal-RN, Brazil. The study was approved by the University Hospital Research Ethics Committee (# 579.479). All subjects provided written informed consent before participation.

Clinical scales

We used the Montgomery–Åsberg Depression Rating Scale (MADRS) to assess depression severity (Montgomery 1979). This scale is composed of 10 items and should be applied by a trained psychiatrist. The sum of the scores can range from 0-60, indicating absence of depressive symptoms (between 0-10), mild depression (11-19), moderate depression (20-34) and severe depression (≥35).

Procedures

Half of the patients received a single dose of 1 ml/kg of ayahuasca adjusted to contain 0.36 mg/kg of DMT and the other half received 1 ml/kg of a placebo. The substance used as placebo was designed to simulate some organoleptic properties of ayahuasca, and to produce low to modest gastrointestinal distress. A single ayahuasca batch was used throughout the study. The batch was prepared and provided free of charge by a branch of the Barquinha church based in the city of Ji-Paraná, Brazil.

Baseline fMRI assessments occurred one day before dosing, and a second fMRI assessment occurred 24h after dosing. MADRS assessments occurred at baseline, one, two and seven days after dosing.

MR Imaging acquisition

fMRI scanning was performed on a 1.5 Tesla scanner (General Electric, HDxt). Functional MRI data were acquired using an EPI sequence with the following parameters: TR = 2000 ms; TE = 35 ms; flip angle = 60 $^{\circ}$; FOV = 24 cm; matrix = 64 x 64; slice thickness = 3 mm; gap = 0.3 mm; number of slices = 35; volumes = 165. T1-weighted images were also acquired using a FSPGR BRAVO sequence with the following parameters: TR = 12.7 ms, TE = 5.3 ms, flip angle = 60 $^{\circ}$, FOV = 24 cm, matrix = 320 x 320; slice thickness = 1.0 mm; number of slices = 128.

Emotional processing protocol

All subjects performed a dry run prior to the fMRI session to assure task compliance. During fMRI scanning, volunteers completed an emotional regulation task adapted from

(Ochsner et al. 2002). Subjects viewed a series of images with different emotional content from the International Affective Picture System (Lang, Bradley, & Cuthbert, 2008). Stimuli were generated using Psychopy (1.79.00 version). Subjects viewed the stimuli with the aid of a mirror placed on the head coil. We selected 72 pictures with negative valence and 36 with neutral valence. Images were classified using valence and arousal scores: negative images had low valence and high arousal scores, and neutral images had medium valence and low arousal scores.

The fMRI emotional task consisted of three conditions: i) passively look at neutral images (NEU), ii) passively look at negative images (NEG), and iii) positive reappraisal of negative images (REAP). During NEU and NEG conditions, participants were instructed to passively view the images presented. For the REAP condition, participants were instructed to reappraise the negative images, trying to reduce their negative impact. Some cognitive reappraisal strategies were explained, such as, thinking that the images were not depicting a real scene, imagining that they were from a movie, or imaging a happy ending for the scene. In addition, we asked the participants to avoid using other emotional regulation strategies, such as closing their eyes or distracting themselves from the image. After presentation of each image, participants were asked to rate their emotional impact using a 5-point Likert scale (very negative, negative, neutral, positive, very positive). Responses were collected via a five-button fiber optic response pad (Pyka com 905 interface da Current Designs, Philadelphia, USA).

Figure 1 shows the fMRI protocol, which followed a block design. In each fMRI session (baseline and after dosing), patients were scanned in three separated runs lasting ~5.5 minutes each, comprising 18 trials, six for each condition. The images used (within and between runs) were balanced with respect to the type of image (NEU ou NEG) and instruction (look or reappraise). Each trial lasted 18 s, beginning by an instruction (LOOK or REAPPRAISE) presented for 2 s, followed by the presentation of an image (NEG or NEU) for 6 s, and by a 6 s rating period, during which volunteers rated the emotional impact of the image using a likert-type scale as very negative, negative, neutral, positive or very positive. After ratting, a fixation cross was presented for 4 s (Fig. 1). Each run contained 18 trials, six for each condition (NEU, NEG and REAPP). The total duration of each run was of 5 min 24 s, and each subject performed two sessions, one at baseline and other at D1, with three runs each.

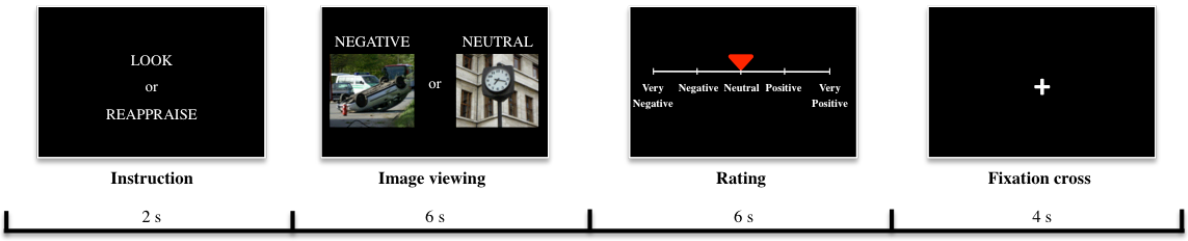

Figure 1 - Representation of a single trial of the fMRI protocol. Each trial was initiated by an instruction (look or reappraise) lasting 2 s, followed by the presentation of an image (NEG or NEU) displayed for 6 s, and by a rating of the emotional impact of the image using a likert-type scale. After ratting, a fixation cross was displayed for 4 s.

fMRI analysis

Data were analyzed using SPM12 (Statistical Parametric Mapping, Wellcome Institute of Cognitive Neurology, Londres, Inglaterra). The first three volumes were discarded to allow for T1 saturation. Preprocessing steps included: head motion correction, slice timing correction, spatial smoothing with an 8-mm FWHM gaussian kernel, and a high-pass filter at 128 s. EPI images were coregistered to each subject's anatomical scan, normalized into a standardized space (Montreal Neurological Institute template) and resampled to 2 mm^3 voxels. Serial autocorrelations were accounted for by a first order autoregressive model (AR1). In addition, motion artifacts were examined using the Artifact Detection Toolbox (ART). Volumes were considered outliers if the global signal deviation was superior to three standard deviations (SDs) from the mean signal, or if the difference in frame displacement between two consecutive volumes exceeded 1 mm. The entire run was excluded if more than 20% of the total volumes were detected as outlier. According to this criterion, four patients had to be excluded from the analysis.

A first-level fixed-effects model was set up for each subject and each session. Five regressors corresponding to the three conditions (NEU, NEG, REAP), instruction, and rating period were modeled using a boxcar convolved with a canonical hemodynamic response function. Periods with the fixation cross were considered as baseline. The model also included the six motion parameters and outlier volumes as regressors of no interest. Images contrasts were calculated using t-statistics for: i) NEG > NEU, ii) REAP > NEU, iii) REAP > NEG.

Contrast images were entered into a second-level random-effects analysis using a flexible factorial design with time as within-subjects factor and treatment as between-subject factor. Whole-brain analysis was performed to assess treatment-by-time interactions. Resulting statistical maps were thresholded at cluster-defining threshold of $p_{CDT} < 0.001$ and a family-wise (FWE) corrected threshold of $p_{cluster\ FWE} < 0.05$.

Region of interest (ROI) analysis

We also conducted a ROI analysis based on previous emotional regulation studies (Hermann et al. 2013; Ochsner et al. 2012). We selected the following ROIs: i) bilateral dorsolateral prefrontal cortex (R-dlPFC and L-dlPFC); ii) dorsomedial prefrontal cortex (dmPFC); iii) bilateral ventrolateral prefrontal cortex (R-vlPFC and L-vlPFC); iv) ventromedial prefrontal cortex (vmPFC); (v) bilateral orbitofrontal cortex (R-OFC and L-OFC); vi) dorsal anterior cingulate cortex (dACC); vii) bilateral subgenual cingulate cortex (R-sgACC and L-sgACC); viii) bilateral amygdala (R-amygdala and L-amygdala); ix) bilateral anterior insula (R-antInsula and L-antInsula); x) nucleus accumbens (NAc).

The dlPFC, dmPFC, vlPFC, and vmPFC masks were provided by Morris and colleagues (Morris et al. 2016). The OFC, amygdala, and NAc masks were obtained directly from the WFU PickAtlas (Maldjian et al. 2003) in SPM12. The dACC mask consisted of a 10 mm sphere centered in x=-8, y=28, z=28 (MNI coordinates) voxel (Hermann et al. 2013). The sgACC mask was based on Brodmann area 25 and subcallosal cingulate cortex from the Harvard-Oxford atlas (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/Atlases). The anterior insula was obtained from a functional atlas (Shirer et al. 2012). Mean beta-values for each ROI were extracted from each subject's contrast images. This was done using the MarsBaR toolbox in SPM12. Repeated-measures ANOVA was used to investigate treatment-by-time interactions. Significance was evaluated at $p<0.05$, two-tailed. Within group post-hoc analyses were per-

formed only in the ayahuasca group, to compute post-acute changes of ayahuasca. We used a paired t-test to examine pre/post differences only in ROIs with significant interaction.

To further investigate the relation between neural changes and the antidepressant effects of ayahuasca, we performed a Pearson's correlation analyses between changes in MADRS scores from baseline to D1, and changes in beta values from baseline to D1 (ΔBeta = Beta-value$_{post}$ – Beta-value$_{pre}$). Again, correlation was performed only in ROIs that showed a significant interaction. Pearson's correlation coefficients were calculated using GraphPad Prism version 7.00 for Mac (GraphPad Software, California, USA).

Results

Behavioral data

Figure 2 illustrates median and max/min ratings obtained in the likert scale for each group and conditions. We used repeated-measures ANOVA for condition (NEU, NEG and REAP) and time (pre and post) as within-subjects factors, and treatment as between-subject factor. Results revealed a significant main effect of condition ($F_{(2,17)}$=62.22, $p<0.0001$), but no effect for time, treatment, or interactions between them. *Post hoc* tests showed lower ratings for the negative than neutral condition ($p<0.0001$), and higher ratings comparing the reappraise and negative conditions ($p<0.01$). These results indicate that the emotional impact of negative images reduced during reappraisal, regardless of time or treatment.

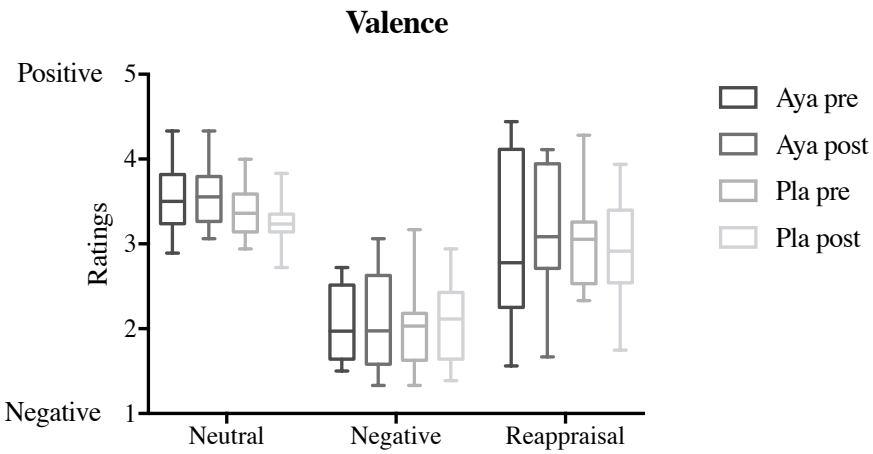

Figure 2. Ratings observed for each condition and both groups (ayahuasca and placebo). Boxplot showing ratings for each condition. Higher scores indicate positive ratings. We observed a main effect of condition ($F_{(2,17)}$=62.22, $p<0.0001$).

Whole brain analysis

No significant interaction between treatment and time was found considering the statistical threshold adopted.

ROI analysis

Figure 3 shows regions with significant treatment-time interaction. We found significant effect in the L-sgACC ($F_{(1,18)}$=4.51, p=0.048) in the NEG > NEU contrast, in the L-vlPFC ($F_{(1,18)}$=8.76, p=0.008) in the REAP > NEU contrast, and in the R-amygdala ($F_{(1,18)}$=4.48, p=0.048), and L-antInsula ($F_{(1,18)}$=4.44, p=0.049) in the REAP > NEG contrast.

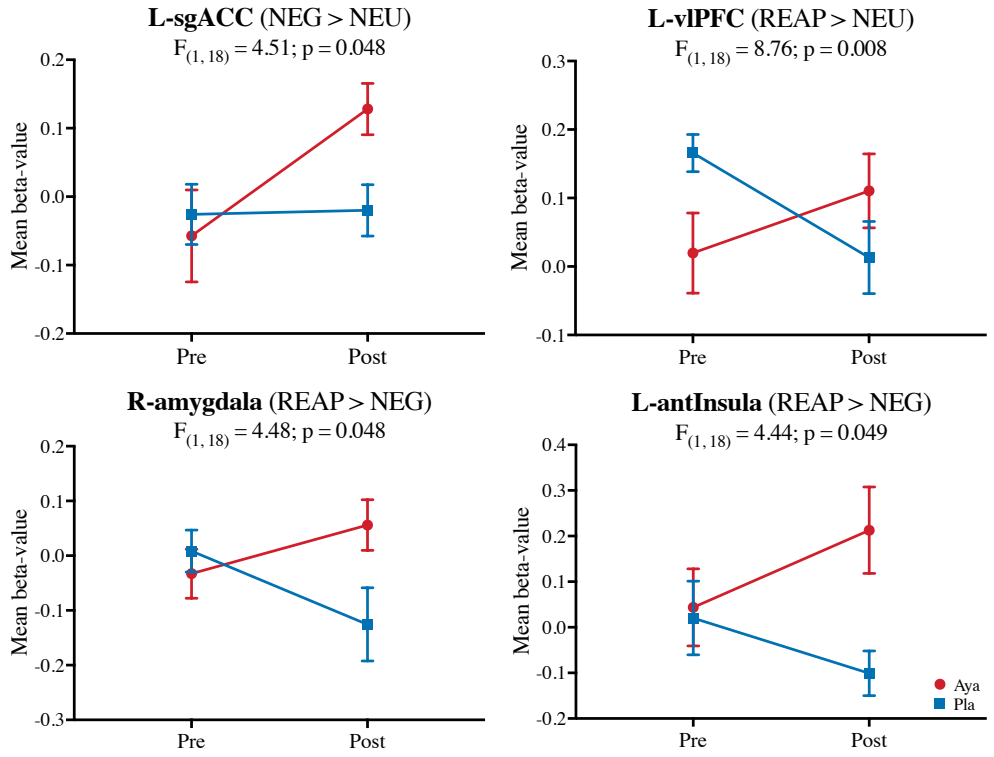

Figure 3 – Significant treatment-by-time interaction effect. Significant effects were found in the L-sgACC ($F_{(1,18)}$=4.51, p=0.048) in the NEG > NEU contrast, in the L-vlPFC ($F_{(1,18)}$=8.76, p=0.008) in the REAP > NEU, and in the R-amygdala ($F_{(1,18)}$=4.48, p=0.048), and L-antInsula ($F_{(1,18)}$=4.44, p=0.049) in the REA > NEG contrast.

Within group - Ayahuasca

Figure 4 shows the within group comparisons, for the ayahuasca group only. We find increased post-acute BOLD signal in the L-sgACC ($t_{(9)}$=2.89, p=0.018) in the NEG > NEU contrast (Fig. 4a). In addition, we observe a positive correlation between the reduction in depressive symptoms and changes in beta-values in the L-sgACC (r=0.75, p=0.012) (Fig. 4b).

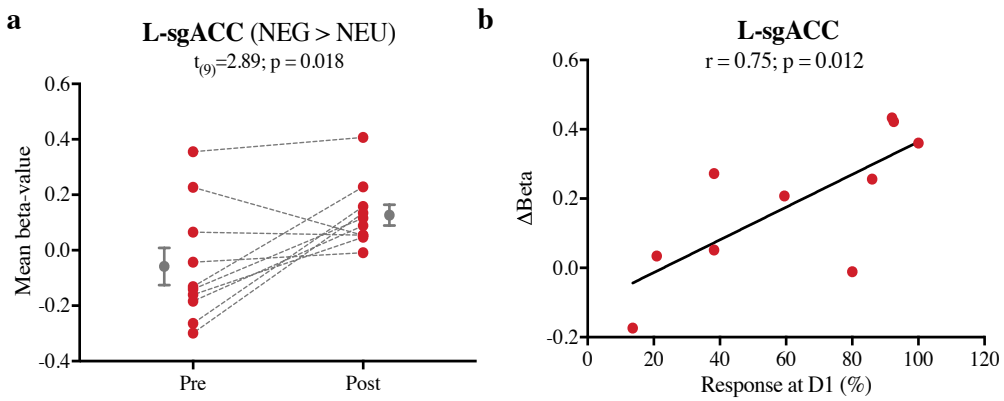

Figure 4 – Within group comparison for the ayahuasca group only. a) Pairwise comparison between pre and post ayahuasca dosing showed increased BOLD signal in the L-sgACC ($t_{(9)}$=2.89, p=0.018), in the NEG > NEU contrast one day after dosing. Gray dots and bars indicate mean and standard error of mean, respectively. **b)** Pearson's correlation between changes in beta-values in L-sgACC from baseline to D1 and changes in MADRS scores from baseline to D1 (r=0.75, p=0.012).

Discussion

This study used fMRI to explore the neural substrates underlying the rapid antidepressant effects of a single dose of ayahuasca in patients with treatment-resistant depression. We found that brain areas involved in emotion processes are differently modulated in patients treated with ayahuasca when compared to placebo. Using an emotional regulation protocol, we observed significant treatment-by-time interactions in the L-sgACC, R-amygdala, L-antInsula, and L-vlPFC regions.

In a recent open-label trial investigating the antidepressant effects of ayahuasca found increased blood perfusion in the subgenual cingulate cortex and anterior insula, 8h after dosing with a single dose of ayahuasca (Sanches et al. 2016). Furthermore, changes in blood flow in the anterior insula, ACC, sgACC and left amygdala were observed in healthy subjects during the acute effect of ayahuasca (Riba et al. 2006). Besides, a recent study reported an increased functional coupling between the rostral part of the ACC and the medial temporal lobe, suggesting that the post-acute effects of ayahuasca are related with changes in the limbic system, which are known to that play an important role in emotion processes (Sampedro et al. 2017).

The sgACC has been implicated in the modulation of emotional behavior (Drevets et al. 2008). This region has been a marker of therapeutic response in depression in several studies following pharmacological, psychotherapy and deep brain stimulation interventions (Delaveau et al. 2011; Mayberg et al. 2005; Siegle et al. 2012). Conversely, we observed increased activity in the L-sgACC activity when passively viewing negative images in the ayahuasca group one day after dosing. Indeed, this finding is in line with previous report from Sanches et al. 2016 that observed increased subgenual activity in patients with depression treated with ayahuasca (Sanches et al. 2016). In addition, these changes in the L-sgACC activity were positively correlated with the reductions in depressive symptoms (MADRS scores) one day after dosing. Most of the studies evaluated correlations between baseline activity from sgACC and clinical outcomes. In our case, we correlate changes in brain activity from baseline to posttreatment with changes in depressive symptoms. In fact, our objective was not to predict treatment outcomes, but rather, understand how subacute changes elicited by ayahuasca relates with its antidepressant effects.

The amygdala is another region frequently implicated with depression. Its hyperactivity is usually associated with depressive symptoms, and its activity is usually modulated by different antidepressant interventions (Godlewska et al. 2012; Murphy et al. 2009; Williams et al. 2015; Sheline et al. 2001). For instance, a single dose of citalopram, a selective serotonin reuptake inhibitor (SSRI), significantly reduced the amygdala response to fearful facial expressions compared to placebo (Murphy et al. 2009). A similar result was observed in another study, the short-term effects seven days of escitalopram use in patients with depression shows reduced activity in the amygdala to fearful faces, compared to placebo (Godlewska et al. 2012). In our study, however, despite the significant interaction effect observed in the right amygdala, no significant changes in amygdala activity were found within the ayahuasca group after dosing.

We also found a significant treatment-by-time interaction effect in the L-antInsula and L-vlPFC, but no within group effects were observed. We also found changes in the anterior insula in a group of patients with treatment resistant depression treated with ayahuasca (Sanches et al. 2016). Previous findings report that hypometabolism in the right anterior in-

sula was associated with remission to cognitive behavior therapy and poor response to escitalopram, while insula hypermetabolism was associated with remission to escitalopram and poor response to cognitive behavior therapy (McGrath et al. 2013). These findings suggest that changes in the anterior insula activity appear to be dependent on the treatment. The modulation of the vlPFC is not consistently observed in depressions, but it is consistently found in emotional regulation tasks (Wager et al. 2008; Ochsner et al. 2012). Classically, the participation of the vlPFC has been implicated in cognitive control and reflexive reorienting (Levy and Wagner 2011). Furthermore, few neuroimaging studies show increased activity of the L-vlPFC in depression (Drevets 2000; Andrews and Thomson 2009). Increased activity in this area in patients with depression viewing negative stimuli when compared with healthy controls (Brody et al. 2001; Brody et al. 1999). Activity in the vlPFC was reduced after treatment with paroxetine, a SSRI (Brody et al. 1999).

Overall, our results indicate that ayahuasca causes subacute changes in regions involved in the emotional processing. Besides, changes in activity in the sgACC are correlated with clinical outcomes in patients with treatment-resistant depression.

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Discussão Geral

Neste trabalho avaliamos os efeitos antidepressivos da ayahuasca em pacientes com depressão resistente ao tratamento, por meio de duas escalas que medem sintomas de depressão (HAM-D e MADRS), utilizando um desenho duplo-cego randomizado, placebo-controlado. A despeito do crescente número de ensaios clínicos avaliando o potencial terapêutico de substâncias psicodélicas, até hoje, nenhum ensaio clínico utilizando esse desenho havia sido realizado para investigar os efeitos antidepressivos desse tipo de substância em uma população de pacientes com depressão resistente ao tratamento. Mostramos ainda que os efeitos psicodélicos agudos e os efeitos antidepressivos da ayahuasca estão correlacionados, de maneira que pacientes que experimentaram efeitos psicodélicos mais intensos durante a sessão de tratamento tiveram maiores reduções nos sintomas de depressão. Também avaliamos esses pacientes utilizando fMRI e vimos que a ayahuasca provoca alterações subagudas em regiões relacionadas ao processamento emocional. Além disso, observamos que essas alterações estão correlacionadas à melhora clínica dos pacientes.

Substâncias psicodélicas, presentes em plantas e fungos, têm sido tradicionalmente utilizadas há séculos em cerimônias religiosas e de cura como parte da cultura de povos antigos. Essas substâncias, que alteram de maneira singular funções da mente e do corpo, sempre foram consideradas sagradas por esses povos, sendo chamadas de "plantas dos deuses" (Schultes and Hofmann 1979).

A pesquisa com psicodélicos, no entanto, surgiu no início da década de 1950, a partir de relatos sobre a potência única e efeitos subjetivos notáveis da dietilamida do ácido lisérgico (LSD, *Lysergic acid diethylamide*)(Carhart-Harris and Goodwin 2017). Em meados da década de 1960 mais de 1000 estudos clínicos, envolvendo mais de 40.000 pacientes, relatavam os efeitos terapêuticos promissores dos psicodélicos (Vollenweider and Kometer 2010). Esses estudos exploravam o uso de LSD, psilocibina, e em menor número, da cetamina, em pacientes com ansiedade, transtorno obsessivo compulsivo (TOC), depressão, disfunção sexual, dependência de álcool, e como alívio na dor e ansiedade de pacientes com câncer terminal (Vollenweider and Kometer 2010, Grinspoon and Bakalar 1979). Apesar de muitas vezes apresentarem resultados positivos, nenhum desses estudos fora conduzido seguindo os recém-promovidos estudos duplo-cego placebo-controlados (Grinspoon and Bakalar 1979, Oram 2012)

No final dos anos 1960, o uso de psicodélicos foi associado fortemente a movimentos de contracultura, culminando com sua criminalização e proibição em 1971, com a declarada 'guerra às drogas' promovida no governo do então presidente americano, Richard Nixon (Nichols 2016). Essas substâncias, pouco tempo antes utilizadas amplamente em estudos clínicos, passaram a fazer parte de uma lista de substâncias ilegais (*schedule I*), sendo consideradas drogas com grande potencial de abuso, sem nenhum uso terapêutico comprovado e não seguras, mesmo sob supervisão médica (Nichols 2016).

Consequentemente, as pesquisas que vinham sendo realizadas sobre os efeitos terapêutico dos psicodélicos em humanos foram severamente prejudicadas, e lentamente desapareceram, deixando diversos caminhos inexplorados e muitas questões em aberto (Vollenweider and Kometer 2010).

Foi apenas no início da década de 1990, após um hiato de mais de 20 anos, que a pesquisa com psicodélicos em humanos ressurgiu (Hermle et al. 1992, Strassman and Qualls 1994, Vollenweider et al. 1997). Também nessa época, surgiram as primeiras organizações científicas, sem fins lucrativos, como a Associação Multidisciplinar de Estudos Psicodélicos (MAPS, *Multidisciplinary Association for Psychedelic Studies*) e o instituto de pesquisa Heffter, que passaram a apoiar e financiar a pesquisa com psicodélicos. De lá para cá, dezenas de estudos de psicofarmacologia, psicologia e neuroimagem utilizando psicodélicos foram realizados (Carhart-Harris and Goodwin 2017).

A retomada dos ensaios clínicos, por sua vez, aconteceu no início dos anos 2000, com um estudo que avaliou a segurança, tolerabilidade e eficácia do uso de psilocibina para tratamento de transtorno obsessivo-compulsivo (TOC) (Moreno et al. 2006). A psilocibina foi associada à redução transitória dos sintomas de TOC em indivíduos com TOC resistente ao tratamento (Moreno et al. 2006). Além disso, já bem recentemente, alguns ensaios abertos passaram a testar a psilocibina no alívio de ansiedade em pacientes terminais de câncer (Grob et al. 2011), no tratamento de dependência a tabaco (Johnson et al. 2014), dependência a álcool (Bogenschutz et al. 2015), e depressão resistente ao tratamento (Carhart-Harris et al. 2016). Em comum, esses estudos reportam segurança, tolerabilidade e melhoras clínicas associadas ao uso da psilocibina. É importante destacar também que em todos esses ensaios clínicos, o uso da psilocibina esteve associado a sessões de psicoterapia.

Recentemente, uma segunda etapa na investigação do uso da psilocibina no tratamento de ansiedade e depressão em pacientes com câncer em estágio avançado foi realizada. Dois estudos duplo-cego, randomizados, placebo-controlados mostraram que a psilocibina associada à psicoterapia é superior ao placebo na redução de sintomas de ansiedade e depressão nesses pacientes (Ross et al. 2016, Griffiths et al. 2016). As melhoras clínicas observadas permaneceram por até seis meses após uma única sessão com psilocibina.

Até agora, apenas um ensaio clínico foi realizado com LSD após o ressurgimento da pesquisa com psicodélicos. Em 2014, o primeiro ensaio duplo-cego, randomizado, placebo-controlado avaliou o efeito do LSD, associado à psicoterapia, no alívio de ansiedade em pacientes com doenças terminais. Comparados ao placebo, os resultados mostram reduções significativas nos escores de uma escala de ansiedade (STAI, *State-Trait Anxiety Inventory*), que permaneceram por até 12 meses (Gasser et al. 2014).

Nesse mesmo período, um grupo de pesquisadores brasileiros passou a investigar o potencial antidepressivo da ayahuasca. Em um primeiro ensaio aberto com 17 pacientes com depressão refratária foi encontrada redução significativa dos sintomas de depressão já 24h após uma única sessão com ayahuasca, que se manteve por até 21 dias (Osório et al. 2015, Sanches et al. 2016). O próximo passo, naturalmente, seria o estudo randomizado, placebo-controlado, que compõe esta tese.

Observamos efeitos antidepressivos significativos da ayahuasca quando comparados ao placebo em todos os instantes de tempo avaliados: um dia, dois dias e sete dias após a sessão de tratamento. Tanto os escores da MADRS quanto da HAM-D se mostraram significativamente menores no grupo que bebeu ayahuasca do que placebo. As taxas de resposta (redução de 50% nos sintomas) foram elevadas para ambos os grupos, ayahuasca e placebo, nos dias D1 e D2. Já sete dias após a sessão de tratamento, a taxa de resposta foi significativamente maior no grupo ayahuasca que no placebo (64% ayahuasca x 27% placebo). Nesse mesmo ponto de avaliação, a taxa de remissão (ausência de sintomas da depressão) foi marginalmente significativa, maior no grupo ayahuasca que no placebo (36% ayahuasca x 7% placebo).

Um ponto que merece destaque é a rapidez dos efeitos antidepressivos que encontramos. Fazendo a avaliação dentro do próprio grupo, observamos efeito significativo já um dia após a intervenção. Por outro lado, os tratamentos medicamentosos disponíveis atualmente,

como SSRI e os tricíclicos demoram em torno de duas semanas para iniciar a resposta terapêutica desejada (Cai, Huang, and Hao 2015, Otte et al. 2016). Nesse aspecto, nossos resultados se comparam aos resultados obtidos com o uso da cetamina, um antagonista de N-metil-D-aspartato (NMDA). A cetamina é frequentemente utilizada como anestésico, e nos últimos anos tem sido testada em pacientes com depressão resistente ao tratamento, tanto unipolar como bipolar. As evidências recentes de ensaios randomizados controlados (RCT, *Randomized Controlled Trial*) mostram potencial antidepressivo rápido da cetamina, com o pico dos efeitos ocorrendo um dia após a administração, e permanecendo significativos por sete dias (Berman et al. 2000, Lapidus et al. 2014, Murrough et al. 2013, Zarate et al. 2006).

É interessante notar que embora tanto a cetamina como a ayahuasca estejam associadas a efeitos antidepressivos rápidos, seus cursos temporais de resposta são diferentes. Estudos com cetamina encontraram o maior tamanho de efeito entre os grupos no D1 (Cohen's d=0.89), diminuindo no D7 (Cohen's d=0.41) (Berman et al. 2000, Lapidus et al. 2014, Murrough et al. 2013, Zarate et al. 2006). Em contraste, os tamanhos de efeitos entre grupos observados no nosso estudo foram menores no D1 (Cohen's d=0.84) e maiores em D7 (Cohen's d=1.49).

Essas diferenças também se refletem na taxa de resposta. No D1, a taxa de resposta da cetamina situa-se entre 37-70% (Berman et al. 2000, Lapidus et al. 2014, Murrough et al. 2013, Zarate et al. 2006), enquanto que no nosso estudo 50% dos pacientes responderam à ayahuasca. No D7, a taxa de resposta da cetamina varia entre 7-35% (Berman et al. 2000, Lapidus et al. 2014, Murrough et al. 2013, Zarate et al. 2006), enquanto no nosso estudo 64% responderam à ayahuasca.

Observamos, ainda, que o efeito placebo observado no nosso estudo é bem superior ao encontrado nos estudos com cetamina (Berman et al. 2000, Lapidus et al. 2014, Murrough et al. 2013, Zarate et al. 2006). Enquanto encontremos taxa de resposta ao placebo de 46% no D1 e 26% no D7, os estudos com cetamina encontram efeito placebo na ordem de 0-6% no D1 e 0-11% no D7.

De forma geral, o efeito placebo que encontramos é bastante alto. É possível que parte da causa esteja associada a características da nossa amostra de pacientes, principalmente em relação a aspectos socioeconômicos. A maioria, senão todos os estudos clínicos que avali-

am efeitos antidepressivos de substâncias psicodélicas avaliam pacientes com status socioeconômicos[10] mais altos que os nossos. Além disso, grande parte dos nossos pacientes viviam sob intenso e constante estresse, em ambientes inseguros, em constante precariedade financeira e social. Para vários deles, participar do nosso estudo era como ficar hospedado em um hotel cinco estrelas. Todos demonstraram satisfação com os cuidados da nossa equipe, ao ponto que muitos expressaram vontade de permanecerem no hospital mais tempo. Esse fator certamente ajudou a elevar o efeito placebo que observamos, e que, na verdade, passamos a chamar no laboratório de "efeito carinho", e não efeito placebo. Outro fator que pode ter influenciado para o alto efeito placebo observado vem do fato que 76% dos pacientes recrutados possuíam algum tipo de transtorno de personalidade, a maioria do cluster B (borderline e histriônico), e evidências sugerem que esses pacientes apresentam maior efeito placebo (Ripoll 2013).

Os mecanismos subjacentes ao efeito antidepressivo rápido que encontramos começam a ser explorados e melhor compreendidos. Do ponto de vista neuroquímico, evidências recentes apoiam os nossos achados. Primeiro, a presença de N,N-DMT, que atua principalmente como agonista serotonérgico e sigma-1 (Smith et al. 1998, Fontanilla et al. 2009, Carbonaro and Gatch 2016, Nichols 2016), estando ambos implicados na fisiopatologia da depressão (Cai, Huang, and Hao 2015, Otte et al. 2016). Observou-se, por exemplo, que a administração de agonistas sigma-1 resulta em efeito antidepressivo, que por sua vez são bloqueados pelo antagonismo de receptores sigma-1. Além disso, sabe-se que os receptores sigma-1 regulam fatores neurotróficos como o BDNF e fator de crescimento nervoso (NGF, N*erve Growth Factor*), proteínas cuja regulação e expressão parecem estar envolvidas na fisiopatologia e no tratamento da depressão (Cai, Huang, and Hao 2015). Em segundo lugar, a presença de MAOi na ayahuasca afeta diretamente os sistemas monoaminérgicos, aumentando a concentração de monoaminas, como serotonina, noradrenalina e dopamina. Isoladamente, a harmina, uma das iMAO contida na ayahuasca, leva à redução de sintomas de depressão em modelos animais de depressão. Por exemplo, a administração de harmina cronicamente em um modelo experimental de depressão (em ratos) levou à redução tempo de imobilidade, aumentou a escalada e o tempo de natação, reverteu a anedonia, aumentou o peso da glândula adrenal, e elevou os níveis de BDNF no hipocampo (Fortunato et al. 2010a, Fortunato et al.

[10]geralmente, inclui a renda, o nível de educação e a profissão

2010b). Da mesma forma, a harmina parece estimular a neurogênese de células progenitoras neurais humanas, derivadas de células tronco pluripotentes, mecanismo também observado em roedores após o tratamento com antidepressivo (Dakic et al. 2016). Em terceiro lugar, a THH funciona como inibidor da recaptação da serotonina (Riba et al. 2003), que é a base de muitos medicamentos antidepressivos como os SSRI.

Além do aumento global na concentração de certos neurotransmissores, como os descritos acima, é possível que parte dos efeitos antidepressivo da ayahuasca tenha origem em mecanismos mais sutis, relacionados a aspectos psicológicos da experiência psicodélica. Assim, pois, investigamos a relação entre os efeitos antidepressivos e os efeitos psicodélicos agudos da ayahuasca, por meio de duas escalas: a HRS e a MEQ30. Encontramos correlações positivas significativas entre a melhora observada dos sintomas de depressão (escores MADRS) e três sub-escalas da HRS: percepção, somestesia e cognição em pacientes que responderam ao tratamento. Dos quatro, cabe destaque para o resultados relacionados às alterações na percepção. De fato, as visões ocupam papel de destaque na experiência psicodélica, ao ponto de receberem nome especial, as *mirações*, como as visões são chamadas por algumas religiões. Em um estudo recente, sugeriu-se que as visões podem desempenhar um papel importante no efeito terapêutico da ayahuasca, pois ajudariam a trazer reflexões pessoais, dar luz a eventos traumáticos, e/ou fornecer *insights* (Frecska et al., 2016).

O conceito de experiência mística foi introduzido pela primeira vez por Abraham Maslow em 1964 (Maslow 1964) como "experiências raras, emocionantes, oceânicas, profundas, e elevadoras que geram uma forma avançada de perceber a realidade, chegando a ser místicas e mágicas". Mais tarde, esse conceito foi aplicado ao campo da ciência psicodélica (Pahnke 1966, Pahnke 1969). Em seu famoso experimento conhecido como "Experiência da Sexta-Feira", Pahnke referiu-se à experiência mística induzida por psicodélicos como sendo "uma experiência que induz um sentido de unidade, de transcendência do tempo e do espaço, um sentimento profundamente positivo, um sentimento de que se está experimento algo sagrado, paradoxal e inefável, que leva a mudanças positivas em diferentes domínios" (Pahnke 1966, Pahnke 1969). Diversos estudos têm sugerido a relação entre os efeitos terapêuticos dos psicodélicos e a experiência mística experimentada: quanto mais intensa a experiência místi-

ca, maiores as melhoras clínicas (Garcia-Romeu, Griffiths, and Johnson 2014, Bogenschutz et al. 2015, Ross et al. 2016, Griffiths et al. 2016).

No nosso caso, observamos uma correlação negativa entre o fator transcendência do tempo e espaço, da MEQ30 e os sintomas de depressão. Devemos notar, que a aplicação da MEQ30 começou após o início da coleta de dados, e que por isso, apenas 15 pacientes foram avaliados por essa escala. Além disso, um fato que nos chama a atenção, novamente, é a diferença dos nosso pacientes com relação aos pacientes dos demais estudos. Enquanto na maioria dos estudos os pacientes haviam tido experiência prévia com substâncias psicodélicas, no nosso caso, todos os pacientes eram completamente "naïves", isto é, nunca tinham experimentado nenhuma substância psicodélica. Portanto, distinguir diferentes aspectos da experiência foi uma tarefa difícil. Mudanças na percepção, como visões, ou na sensação corporal, podiam facilmente serem confundidas com efeitos mais subjetivos e sutis, como a sensação de unidade com o universo ou a transcendência do tempo e do espaço. Por último, outro fator de confusão em relação a MEQ30, pode ter sido o baixo grau de instrução formal dos nossos pacientes. A MEQ30 é composta por questões que utiliza palavras e conceitos bastante específicos, o que pode ter dificultado o entendimento por parte dos pacientes.

A maior parte dos estudos que utilizaram a MEQ30 sugere que a relação entre MEQ30 e melhora clínica é bastante específica o que implica na inexistência de relação entre a intensidade dos efeitos psicodélicos, como por exemplo aqueles medidos pela HRS, e a melhora clínica. Nos nossos dados, no entanto, encontramos uma forte correlação entre alterações na percepção e a redução nos sintomas de depressão, sugerindo que outras dimensões, além da experiência mística, pode ter um papel importante nos benefícios terapêuticos dos psicodélicos.

Outro aspecto que investigamos foi se a ayahuasca causa alterações subagudas em regiões cerebrais relacionadas ao processamento emocional, e como essas possíveis alterações se correlacionam com os efeitos antidepressivos observados. De maneira geral, observamos mudanças na amígdala, ínsula anterior, no córtex ventrolateral e na porção subgenual do giro do cíngulo anterior (sgACC). Particularmente, variações no sinal do sgACC um dia após a sessão de tratamento, foram correlacionadas positivamente à redução nos sintomas de depressão, também um dia após o tratamento.

A continuidade desse projeto segue agora dois caminhos. O primeiro diz respeito à análise de outros dados adquiridos. Por exemplo, a coleta de dados das escalas de depressão (HAM-D e MADRS) foi feita mensalmente, durante seis meses após a sessão de tratamento. Apesar de não termos conseguido acompanhar todos os pacientes que fizeram parte da primeira semana do experimento, é interessante investigar o que acontece com o efeito antidepressivo após os sete dias. Além disso, os dados das outras avaliações (neuropsicológica, sono, bioquímica, etc...) encontram-se em fase de análise, o que abre a perspectiva tanto de compreender os mecanismos dos efeitos antidepressivos da ayahuasca, como a possibilidade de realizar análises multifatoriais, em que as diferentes medidas serão utilizadas como fatores.

O segundo caminho é a volta do projeto a Ribeirão Preto. Nessa nova fase, sob a coordenação do Prof. Jaime Hallak da Faculdade de Medicina de Ribeirão Preto/USP, o estudo irá avaliar a resposta antidepressiva da ayahuasca em um esquema de tratamento, e não mais dose única.

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Apêndice

APÊNDICE A – Capítulo publicado durante o doutorado

Chapter 2

The Therapeutic Potentials of Ayahuasca in the Treatment of Depression

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Abstract Major depressive disorder (MDD) is generally classified as a mood disorder with a profound effect on the individual's behavior and quality of life. According to the World Health Organization, in about 20 years, depression will be the disorder with the most significant repercussions, both socially and economically. Despite the substantial progress in the development of new antidepressants, their effectiveness remains low, with remission of about 50 % after a single regime of treatment. The most common form of pharmacological treatment of MDD is based on selective serotonin reuptake inhibitors (SSRIs), designed to increase extracellular levels of the neurotransmitter serotonin. Unfortunately, antidepressants currently available based on SSRIs may take several weeks to achieve the desired therapeutic effects.

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B. C. Labate and C. Cavnar (eds.), *The Therapeutic Use of Ayahuasca*,
DOI: 10.1007/978-3-642-40426-9_2, Ó Springer-Verlag Berlin Heidelberg 2014

Therefore, massive effort has been devoted to find alternative treatments for MDD. For example, the use of ketamine, of (±)-1-(2,5-Dimethoxy-4-iodophenyl)-2- aminopropane (DOI), and b-carbolines is under current investigation. Based on evidence from the literature and a pilot study conducted by our group, we speculate about the possible therapeutic potential of ayahuasca for MDD. In part, such con- jecture is based on the fact that ayahuasca combines N,N-dimethyltryptamine (DMT), acting particularly on serotonin neurotransmission through 5-HT_{2A} recep- tors and monoamine oxidase inhibitors (MAOI), both involved, at least indirectly, with pharmacological formulations intended for MDD treatment. In this chapter, we will review the major aspects of MDD such as diagnosis, current pharmacological treatments, and the motivations to use ayahuasca as a novel alternative.

Major Depressive Disorder: Causes and Diagnosis

Major depressive disorder (MDD) is generally classified as a mood disorder with a profound effect on the individual's behavior and quality of life. It has a prevalence of 17 % throughout life, being twice as common in women as it is in men and usually begins in the third decade of life. For most people, MDD presents itself in recurrent episodes. However, about 20–25 % of patients are chronically ill. According to the World Health Organization (WHO), MDD is the fourth leading cause of morbidity, and they predict that by 2020, it will be ranked second disease burden worldwide (Fava and Kendler 2000).

MDD is associated with intense personal suffering, high morbidity, and increased mortality (Ebmeier et al. 2006). The Diagnostic and Statistical Manual of Mental Disorders of the American Psychiatric Association (DSM-IV-TR) defines this condition based on the presence of depressed mood (irritable mood in children and adolescents) and/or loss of interest or pleasure (anhedonia) for at least 2 weeks, accompanied by at least four of the following symptoms: (a) considerable change in weight (5 % of body weight), (b) frequent insomnia or hypersomnia, (c) psychomotor agitation or retardation, (d) fatigue or loss of energy, (e) low self-esteem or inappropriate guilt, (f) diminished capacity to think, concentrate, or make decisions, (g) recurrent thoughts of death, suicidal ideation, or suicide attempts. In addition, the diagnosis requires that the symptoms cause significant distress and/or impairment in social, occupational, or other areas of life, and should not be directly caused by another general medical condition or psychoactive substance use and must not meet the criteria for mixed episode (in which the diagnostic criteria of depression and mania occur concurrently) (American Psychiatric Association [APA] 2000b).

Multiple theories attempt to explain the etiology of depression, but the most widely accepted one is the monoamine hypothesis. This theory suggests that MDD is a result of decreased brain levels of monoamines, such as dopamine, norepi- nephrine, and serotonin (Wong and Licinio 2001). Among these, serotonin has received the most attention. Besides the reduction

of serotonin levels found in depression, studies also point to an altered expression of $5HT_{1A}$ autoreceptors and heteroreceptors. The most consistent finding is an increase in pre-synaptic $5HT_{1A}$ autoreceptors, which inhibit the release of serotonin, and consequently reduce serotonin levels in the synaptic cleft. A reduced number of postsynaptic $5HT_{1A}$ heteroreceptors in the hippocampus and prefrontal cortex, presumably induced by high cortisol levels also found in these patients, is also associated with MDD.

On the other hand, the monoamine hypothesis does not explain important matters such as the causes of the monoaminergic disturbance and the elevated refractoriness to the treatment of MDD with antidepressant drugs that target the increase of the monoamine levels in the synaptic cleft. In this scenario, several alternative hypotheses were brought to focus such as hypothalamic–pituitary–adrenal (HPA) axis dysfunctions and the inflammatory and neurodegenerative hypotheses.

One of the most common HPA abnormalities observed in depressed patients is an increase in reactivity of this axis. Scientific evidence indicates that MDD patients have impaired glucocorticoid receptor (GR) function, which results in reduced negative feedback in the HPA system, leading to chronically high levels of adrenocorticotrophic hormone-releasing factor (CRF), and increased cortisol in the plasma, urine, and cerebrospinal fluid (Zunszain et al. 2011).

Complementarily, the inflammatory theory is based on the strong mutual regulation and communication between the immune and HPA systems (Leuchter et al. 2010). Chronic activation of the HPA axis also provokes GR resistance in immune cells (Leonard 2007). Normally, basal cortisol induces the production of lymphocytes T CD4 Th2, but chronicle GR resistance leads to an imbalance in the immune system, displacing the production of these lymphocytes to a Th1 subtype, thus reducing the concentration of anti-inflammatory cytokines (IL-4, IL-10), and increasing the pro-inflammatory cytokines (IL-1, IL-6, TNF-a) (Leuchter et al. 2010; Li et al. 2011). In turn, the massive liberation of pro-inflammatory cytokines induces a decrease in GR function (Pace et al. 2007).

Taken together, a link between these three theories has been observed: The excess of circulating corticosteroids and pro-inflammatory cytokines increases activity of indoleamine 2,3-dioxygenase (IDO), an enzyme which induces for- mation of kynurenine in the tryptophan-kynurenine pathway, resulting in the deficiency of serotonin commonly observed in depression (Miura et al. 2008).

The neurodegenerative hypothesis is based on the fact that many depressed patients have reduced hippocampal volume (Bremner et al. 2000). Studies suggest that such reduction does not come only as a consequence of neuronal death (Sapolsky 2004). Instead, it can be a result of a reduction in neurogenesis induced by high glucocorticoid, pro-inflammatory cytokine levels and reduced serotonin availability, which in turn can inhibit the production of cell growth factors such as brain derived neurotropic factor (BDNF) (Shimizu et al. 2003). Animal models of depression (Berry et al. 2012) have shown that social deprivation in mice leads to increased depressive-like behaviors, elevated corticosterone levels, and reduced BDNF levels (Zhou et al. 2008).

Despite the dysregulation of the inflammatory proteins, stress hormones, and brain

factors associated with depression, one of the main challenges regarding MDD diagnosis is the absence of a specific biomarker related to this disease. The use of biomarkers has an important role in understanding and monitoring conventional and alternative treatments (Leuchter et al. 2010). The current consensus is that no biomarker alone is sufficient to predict and identify individuals at risk, make the diagnosis, or direct clinical treatment decisions concerning depression. Accordingly, the construction of a multimodal panel of biomarkers is recommended to obtain the accuracy required in clinical practice or research protocols (Lakhan et al. 2010). For MDD, there are several candidates for identifying bio- markers, including neuropsychological assessment, electroencephalography (EEG), polysomnography (PSG), biochemical markers, medical imaging, genomics, proteomics, and metabolomics, among others.

Biochemical Markers

Information on biochemical markers is accessed indirectly by measurements of the cerebrospinal fluid (CSF), saliva, or blood. In this scenario, CSF may be considered the source that best reflects brain activity. However, it is not easily accessible on a risk-free basis. On the other hand, although urine and feces are easily collected, it is difficult to associate levels of metabolites present in these two vehicles with the ones found in the brain. Saliva has also been used with success for the measurement of steroid hormones, such as cortisol (Dziurkowska et al. 2013). However, this is generally not considered a reliable strategy for use with depressed patients (Knorr et al. 2010). Therefore, biochemical markers have been sought in the blood, both for its easy accessibility and for the fact that many molecules found in the brain are excreted via the route of blood circulation.

Curiously, plasmatic factors which target the serotoninergic system are not considered good biomarkers for chronic depression (Pivac et al. 2002). On the other hand, high concentration of cortisol in the plasma has frequently been read as a biomarker indicative of depression, in agreement with the hypercortisolemia theory (Leonard 2007; Tadic´ et al. 2011). Moreover, plasmatic pro-inflammatory cytokine levels, which are reported to be increased in MDD, are also considered reliable biomarkers, in accordance with inflammatory theory (Li et al. 2011).

Polysomnographic Markers

Sleep abnormalities are consistently revealed in EEG findings in patients suffering from MDD. Although there is no single marker of sleep specifically associated with depression, many studies have found differences in sleep between MDD patients and healthy control subjects. The use of polysomnography (PSG) in these patients has revealed impaired sleep continuity, reduced latency to REM sleep (interval between sleep onset and the occurrence of the first REM period), increased amount of REM sleep, longer first REM period, increased REM density (number of eye movements during REM), and reduced slow-wave sleep (Benca et al. 1992; Riemann et al. 2001; Tsuno et al. 2005).

The reduction in the latency to REM sleep associated with depression has been observed

since the 1970s. Several studies have reported that this change is related to the secretion of cortisol at night, suggesting that dysregulation of mood, sleep, and HPA may be interconnected (Poland et al. 1992). An inverse relationship is observed between cortisol level and the latency shortening: Subjects showing the shortest REM latency also have the greatest degree of HPA activity (Asnis et al. 1983). Asnis and colleagues speculate that the association between REM latency and the HPA axis is caused by a dysregulation of the muscarinic cholinergic system, which exerts a role in both physiologic systems (Asnis et al. 1983).

In addition, it has been proposed that the decreased latency to REM sleep associated with depression may be a familiar trait (Giles et al. 1987). Giles and her co-researchers investigated risk factors in patients with a lifetime history of depression. They reported that the relative risk for MDD in relatives with reduced REM latency was almost three times greater than for relatives with non-reduced REM latency (Giles et al. 1988). They also found shortened REM latency, even in psychiatrically asymptomatic first-degree relatives of depressed probands (Giles et al. 1989). Other investigators reported sleep disorders (Lauer et al. 1995) and dysregulation of the HPA axis (Mannie et al. 2007) in otherwise healthy adults, but whose first-degree relatives had a history of depressive disorder.

Furthermore, the connection between REM sleep and depression has been demonstrated by the fact that acute sleep deprivation (total, partial, or specifically REM sleep) alleviates depressive symptoms (Benca et al. 1992; Riemann et al. 2001). Pharmacological treatment also influences REM sleep. It has been shown that antidepressants decrease the amount of REM sleep. In addition, such changes in the initial stages of antidepressant treatment are a predictor of treatment out- come (Riemann et al. 2001).

Magnetic Resonance Imaging Markers

The use of magnetic resonance imaging has helped to identify the psychopathological mechanisms underlying MDD. Recent studies show that depressed patients present neuroanatomical and functional alterations when compared to healthy controls. One of the most consistent results is the reduction of hippocampal volume in MDD (Bremner et al. 2000; Lorenzetti et al. 2009; MacQueen 2009). Another common finding is the volume reduction in some of the basal ganglia structures, such as the caudate and putamen (Lorenzetti et al. 2009). Although the results are not always convergent, such morphological changes are an indication that structural images can provide information relevant to the characterization of depression.

In addition to brain anatomy changes, some studies used functional neuroimaging techniques to evaluate the brain function of MDD patients. The amygdala, a medial temporal lobe structure that is highly sensitive to emotional stimuli, has been repeatedly implicated in depression symptoms. Studies using functional magnetic resonance imaging (fMRI) showed hyperactivity of the amygdala of depressed patients (Davey et al. 2011; Sheline et al. 2001).

Moreover, the ventromedial prefrontal cortex, the anterior cingulate cortex, and the inferior parietal cortex have been the subject of many studies on depression. These regions constitute a network known as the Default Mode Network (DMN), which is characterized by greater activity during rest than during the execution of a goal- specific task (Raichle et al.

2001). The DMN has been implicated in processes involving self-judgments, recall of autobiographical memories, mental simulations, mind-wandering, and daydreaming (Buckner et al. 2008; Northoff et al. 2006). Using an emotional regulation task, Sheline and colleagues examined the functionality of the DMN in patients with MDD, investigating whether the ability to regulate its activity, and therefore the self-referential processing, was impaired. The results showed that the individuals with depression failed to modulate different regions of the DMN, including the anterior cingulate cortex, lateral parietal cortex, the medial prefrontal cortex, and the lateral temporal cortex. Furthermore, some studies showed alterations in the functional connectivity patterns of the DMN. For instance, Greicius et al. (2007) reported a significant increase in functional connectivity in the subgenual cingulate cortex and precuneus in depressed patients when compared with healthy controls. These results are consistent with clinical symptoms, such as rumination, in which MDD patients are impulsively focused on the past and/or future negative consequences, rather than reflecting on the present moment (Devilbiss et al. 2012).

Abnormalities in functional connectivity of other networks were also found in depressive patients (Anand et al. 2005; Sheline et al. 2010; Veer et al. 2010). For example, Veer and colleagues showed that there is: (1) a decrease in connectivity between the amygdala and the left anterior insula, structures related to affection; (2) reduction in the connectivity of the left frontal pole, a network associated with attention and working memory; and (3) a decrease in bilateral connectivity of lingual gyrus and ventromedial visual areas (Veer et al. 2010). The connectivity has also been related to the response to antidepressant treatment. A study by Anand et al. (2005), evaluated the effect of sertraline, a commonly used antidepressant, in cortico-limbic connectivity. After treatment, patients showed increased connectivity between the anterior cingulate cortex and the limbic regions, a circuit that has been linked to emotional regulation.

Pharmacological Treatments

Several alternatives are available for treatment of MDD. The most common ones are the use of antidepressant medications (Uppal et al. 2010), psychotherapy (Cuijpers et al. 2011), electroconvulsive therapy (Andrade et al. 2010), and other somatic therapies (Segal et al. 2010). The goal of treatment is the remission of the depressive episodes, defined by the DSM-IV as the absence of MDD symptoms for at least 2 months (Kennedy et al. 2001). A treatment response is considered when depressive symptoms are reduced by 50 % or more, usually evaluated by the Hamilton Scale (HAM-D), and not necessarily mean remission (Ebmeier et al. 2006).

In general, pharmacological treatment has a number of phases. The first, known as acute, lasts about 8 weeks, and is essential to evaluate the patient's response to the treatment (Bolland and Keller 2004). If this first step is successful, there is another period that lasts 16–20 weeks and aims to prevent possible recurrence of depressive episodes (Kennedy et al. 2001). Some cases go through the maintenance phase indefinitely, for example, individuals at high risk of recurrence, such as those who have had multiple episodes or partial response to treatment, and patients who have episodes of high severity with psychotic symptoms and suicidal risk (APA 2000a).

The discovery of antidepressants in the 1950s drastically transformed the treatment of depression, and they remain the leading strategy (Ebmeier et al. 2006). Several classes of antidepressants are available, classified according to their chemical structure, effect on the synapses, and action on reuptake and metabolism of neurotransmitters.

The first generation of antidepressants can be divided into tricyclics (TCA) and monoamine oxidase inhibitors (MAOI). Both act by increasing extracellular levels of monoamines; TCA by inhibiting the reuptake of dopamine, noradrenaline, and serotonin, and MAOI by preventing the action of MAO, thereby avoiding the degradation of monoamine neurotransmitters (Bolland and Keller 2004).

In addition to the therapeutic action, TCA acts on several other receptors, leading to antimuscarinic, antihistaminic, and anti-a2 adrenergic effects that lead to undesirable outcomes such as urinary retention, constipation, orthostatic hypotension, weight gain, and somnolence. Furthermore, TCA block sodium channels interfering with nerve conduction, becoming potentially arrhythmogenic. Although recently questioned, it is classically believed that the main side effect of MAOI is the high risk of hypertensive crisis triggered by its combined ingestion with foods containing tyramine, a sympathomimetic amine, which is metabolized by MAO (Grady and Stahl 2012).

Given the low selectivity of classic antidepressants, newer antidepressants were developed. Among these are the selective serotonin reuptake inhibitors: SSRIs (fluoxetine, paroxetine, etc.), selective noradrenaline reuptake inhibitors (reboxetine), and serotonin and norepinephrine reuptake inhibitors (venlafaxine, duloxetine, and desvenlafaxine). Moreover, there are antidepressants with multiple action mechanisms, such as mirtazapine, which acts as a noradrenergic pre-synaptic a2- receptor antagonist and serotonin ($5HT_2$ and $5HT_3$) antagonist, and nefazodone which act by inhibiting the reuptake of serotonin and norepinephrine, and as a 5HT and a2 antagonist (Cipriani et al. 2009).

The newer antidepressants, such as SSRIs, have essentially the same mechanism of action as the first generation, i.e., they increase the level of monoamines in the brain (Zarate 2011; Zarate et al. 2006). However, this new generation offers more safety. Side effects, although milder, are still present, and are specific to different classes of medications. For example, sexual dysfunction and gastrointestinal disorders are common with the use of SSRIs, and sleepiness and weight gain are associated with the use of mirtazapine (Cipriani et al. 2009). Given that the effectiveness of the various antidepressants is similar, the choice of medication is made based on side effects, safety, tolerability, patient's individual preferences, quantity and quality of clinical trial data, and cost (APA 2000a).

With respect to the modulation on the HPA axis made by different antidepressants, evidence indicates that it depends on the type of antidepressant and length of treatment. Increased adrenocorticotropic hormone (ACTH) and cortisol levels have been reported 5 hours after a single oral administration of reboxetine, and a decrease of these hormones after a single oral administration of mirtazapine in both healthy individuals and in subjects with depression (Schüle 2006). Moreover, a gradual and significant reduction of HPA activity has been found after 5 weeks of treatment with reboxetine in depressed patients (Schüle et al. 2006). On the other hand, treatment with mirtazapine only reduces HPA activity during the first week; after

that, HPA activity increases again (Pariante et al. 2004).

Despite substantial progress in the development of new drugs, less than 50 % of patients achieve remission as a result of antidepressant use, even after four different pharmacological treatment regimes (Warden et al. 2007). Besides low effectiveness, the pharmacological treatments that are currently available carry another limitation associated with the chronology of the drug action: It takes several weeks to reach the desired therapeutic effects (Zarate 2011; Zarate et al. 2006). Thus, enormous effort has been devoted to the search for alternative pharmacological treatments that could improve treatment efficiency and accelerate the onset of therapeutic effects (Uppal et al. 2010). For instance, studies conducted with ketamine, an NMDA antagonist, showed antidepressant effects after a single intravenous injection, which persisted significantly after 1 week (Liebrenz et al. 2007). Furthermore, the use of (±)-1-(2,5-Dimethoxy-4-iodophenyl)-2-amino- propane (DOI), a powerful $5HT_{2A/2C}$ agonist, resulted in positive responses in two models of anxiety in mice (Dhonnchadha et al. 2003). In another study, mice subjected to the forced swimming test, a common paradigm used to study depression in rodents, exhibited decreased immobility time 6 h after receiving DOI, an effect that is considered predictive of antidepressant response (Masuda and Sugiyama 2000).

Another serotonergic agonist, N,N-dimethyltryptamine (DMT), has also been considered a potential antidepressant. Some hypotheses have emerged that, at low doses, the anxiolytic mechanism associated with DMT would be mediated by a trace amine receptor, which could be one of the sites of action of endogenous DMT (Jacob and Presti 2005).

Anxiolytic and antidepressant effects are also related to β-carbolines. In a recent study, the use of harmane, norharmane, and harmine in an animal model was capable of reducing the time of immobility in the forced swimming test (Farzin and Mansouri 2005). Similar effects were observed in animal models using the elevated plus maze test, a common paradigm for the study of anxiety in rodents (Aricioglu and Altunbas 2003). It has recently been found that the use of harmine alone in rodent models leads to the reduction of various signs and symptoms associated with depression, such as anhedonia, and regulation to normal levels of ACTH and hippocampal BDNF (Fortunato et al. 2010).

Based on the evidence presented here, and in a pilot study conducted by our group, described below, the question arose regarding the use of substances that combine DMT and MAOI, as is the case of ayahuasca, in MDD treatment. There are several anecdotal reports that the ritual use of ayahuasca is associated with the relief of depression symptoms.

Ayahuasca tea is traditionally prepared by decoction of the bark and stem of the Banisteriopsis caapi vine with leaves of the Psychotria viridis bush. The B. caapi contains the alkaloids harmine, tetrahydroharmine (THH), and harmaline; the three belonging to the b-carbolines group, and corresponding to 0.05–1.95 % of the dry weight of the plant. In addition, P. viridis provides the hallucinogenic tryptamine DMT, which corresponds to 0.1–0.66 % of the dry weight of the plant (McKenna et al. 1984; Riba et al. 2003).

Ayahuasca's activity is dependent on the synergistic interaction between these components. β-carbolines are potent reversible and competitive MAOI (Mckenna et al. 1984) and can increase serotonin levels, blocking its deamination. Their main action in ayahuasca is apparently to protect DMT from peripheral degradation, preventing oxidative deamination of

the orally ingested DMT and enabling it to reach the central nervous system (McKenna 2004). The pharmacological effects of DMT also depend on its interaction with the serotoninergic system. DMT is the substrate for both cell-surface serotonin uptake transporters (SERTs) and neuronal vesicle monoamine transporters (VMAT2). Unlike drugs that are uptake inhibitors, DMT is transported into the cytosol or vesicle by SERT or VMAT2, respectively (Cozzi et al. 2009). Therefore, high intracellular and vesicular concentrations of DMT may be achieved inside of neurons, and can interact with intracellular sigma- 1 receptors located in the mitochondria-associated endoplasmic reticulum membrane (Su et al. 2009). Hence, the DMT can be released into the synaptic cleft upon vesicular fusion to interact with cell-surface sigma-1 receptors or serotonin post- synaptic receptors (Cozzi et al. 2009).

The effects of ayahuasca are heterogeneous (Riba et al. 2001), and encompass sensory, cognitive, and affective changes (Prado et al. 2009), rich visual experiences (de Araujo et al. 2011), and entheogenic experiences (Shanon 2003). These effects begin between 35 and 40 min after tea ingestion, reaching their height between 90 and 120 min, and lasting for 4 h (Riba et al. 2003). Thus far, all studies to date have demonstrated the safety of ayahuasca, with reports of individuals who have used it for more than 30 years without evidence of harm to health (Callaway et al. 1999; Grob et al. 1996; Riba et al. 2001; Riba et al. 2003). Changes in both blood pressure—systolic, diastolic, and mean—and heart rate are not significant (Riba et al. 2003). Furthermore, a recent study showed that the ritual use of the tea is not associated with the psychosocial problems that are usually found with other drugs (Fábregas et al. 2010).

Preliminary evidence of its potential use as an antidepressant is encouraging. A double blind, placebo controlled experiment indicated that the use of ayahuasca significantly reduces the scores of behavioral scales of panic and hopelessness in participants who were under the influence of the tea compared to a control group (Santos et al. 2007).

Ayahuasca and MDD Biomarkers

In addition to the pharmacological evidence described previously, other evidence might also support the use of ayahuasca as an antidepressant. Recent studies have indicated that regular ayahuasca use is involved in long-term modulation of the serotonin systems in the brain, specifically in SERTs levels. Such modulation can increase the serotoninergic system's function, which might be a mechanism for its possible positive effect in depressed patients. Ayahuasca also has significant effects on the endocrine system, mainly in the HPA axis, and the immune system. Studies have found increases in prolactin and cortisol levels approximately 2 hours after a single ayahuasca dose. This increase of cortisol has an impact on cell immunity and reduces CD3 and CD4 lymphocytes after ayahuasca use (Dos Santos et al. 2011). Another study used two sequential doses of ayahuasca with an interval of 4 hours and found endocrine and immunomodulatory effects analogous to those previously reported (Dos Santos et al. 2012). In line with this evidence, rapid increase in cortisol levels was observed in a single acute treatment with antidepressants like reboxetine that act on the serotoninergic system (Schüle 2006).

Barbanoj and colleagues investigated the effect of ayahuasca on sleep. They evaluated sleep quality, polysomnography, and spectral analysis in 22 healthy volunteers following a

unique ayahuasca dose administration during the day. Results indicated that ayahuasca had no significant effects on sleep initiation or continuity as assessed by subjective and objective measures. Furthermore, it was found that the tea inhibits REM sleep, decreasing its duration in absolute values and in percentage of REM sleep. A trend increase in REM latency was also reported (Barbanoj et al. 2008). Based on these results, one can further speculate that ayahuasca might have therapeutic potential, as evidence from the literature points out that PSG changes in depression (increased amount of REM sleep and reduction in REM latency) go in the opposite direction from the changes induced by ayahuasca (Benca et al. 1992; Riemann et al. 2001; Tsuno et al. 2005).

There is also fMRI evidence of the potential antidepressant effects of ayahuasca. In a study conducted by our group, which aimed at evaluating the changes induced by ayahuasca in the DMN, 10 healthy subjects submitted to two fMRI sessions: one before and one right after tea intake. It was observed that ayahuasca caused a reduction in the fMRI signal of central nodes of the DMN, such as the anterior cingulate cortex, the medial prefrontal cortex, the posterior cingulate cortex, the precuneus, and the inferior parietal lobe. Moreover, changes in connectivity patterns of the DMN were observed (Fig. 2.1), especially a decrease in the functional connectivity of the precuneus (Fontes et al. 2012). However, some studies show an opposite modulation in patients with depression, i.e., increased DMN activity and functional connectivity (Greicius et al. 2007; Sheline et al. 2009).

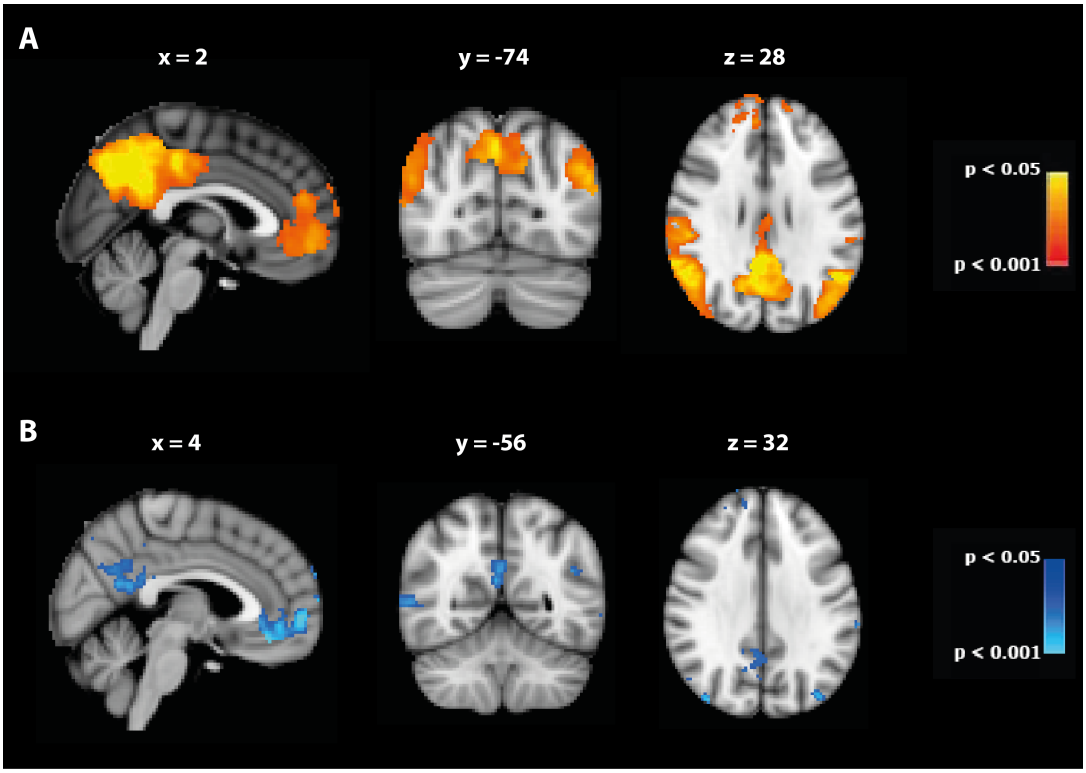

Fig. 2.1 Ayahuasca effects on the DMN. **a** An fMRI image showing the set of regions that comprise the DMN. **b** Regions where ayahuasca caused a BOLD signal reduction. Statistical maps were obtained comparing the groups before and after ayahuasca intake ($p<0.05$ uncorrected)

Our group has been conducting an exploratory study of the feasibility of the use of ayahuasca as an antidepressant. The ethics committee of the Clinical Hospital of Ribeirao Preto approved this study (No. 2484/2008). Preliminary evaluations were conducted in three female subjects with a clinical diagnosis of recurrent depressive disorder and current mild to severe depressive episodes without psychotic symptoms. The subjects were in the washout period between medication changes, and had been without antidepressant medication for 2 weeks.

Patients received a single oral dose of 2 ml/Kg of ayahuasca tea and were assessed using psychiatric scales, including the HAM-D, 10 min before administration of tea, and 40, 80, 140, and 180 min after ingestion, and on days 1, 2, 7, 14, and 28 after the experimental session. Results showed a significant decrease of HAM-D scores over time, beginning at 40 min after intake and lasting for 14 days (de Lima Osório et al. 2011).

It is interesting to notice that the improvement observed in HAM-D scale can also be further corroborated by the patient's testimonials. One patient stated: ''I stayed huddled and crying softly, I was completely huddled and I could not answer because I felt as if the devil and Our Lady were battling for my soul and I could not interfere. After a long battle Our Lady won and pulled me to her side and I felt an intense joy.'' Another said: ''I felt as if I was trying to hold a ball of blue energy. I did not succeed because it was like my hands were different poles of a magnet, moving them apart. When I finally got to hold it, the energy came over me and it all made sense. From then on, I was no longer feeling depressed.''

Although this evidence must be considered with caution due to the inherent limitations of exploratory studies, the results suggest that ayahuasca has antidepressant properties presenting an interesting acute effect.

Final Remarks

New pharmacological approaches to MDD treatment have expanded beyond the model of serotonin reuptake, including the use of ketamine as well as the potential use of novel substances such as ayahuasca. Different aspects related to the identification of biomarkers and theories about the psychophysiological action of ayahuasca were characterized and presented as part of the zeitgeist of depression. Although the evidence presented herein was supported by international studies, more research is necessary to support the pharmacological use of ayahuasca and of similar DMT/MAOI combinations in the treatment of MDD. Based on that evidence, and on studies conducted by our group, it is possible to glimpse promising pharmacological implications for the use of ayahuasca in treating depression.

There is an interesting aspect to consider about the use of ayahuasca in depression: A closer look at our preliminary results reveals that the depressive signs and symptoms are reduced after a single ayahuasca intake, and that the effects last for about 14 days. Curiously, in the context of the Brazilian ayahuasca religions, the interval between sessions is exactly 2 weeks. One must also consider the number of anecdotal reports from experienced ayahuasca drinkers, and also from naïve ayahuasca drinkers, supporting the notion that ayahuasca has antidepressant potentials. From a broader reflection on how knowledge is transformed, the discovery of ayahuasca's antidepressant effects might be considered a thought- provoking

example of a successful dialog between science and cultural tradition.

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APÊNDICE B – Artigo publicado durante o doutorado

SCIENTIFIC REPORTS

OPEN

Shannon entropy of brain functional complex networks under the influence of the psychedelic Ayahuasca

Received: 1 November 2016
Accepted: 20 June 2017
Published online: 07 August 2017

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The entropic brain hypothesis holds that the key facts concerning psychedelics are partially explained in terms of increased entropy of the brain's functional connectivity. Ayahuasca is a psychedelic beverage of Amazonian indigenous origin with legal status in Brazil in religious and scientific settings. In this context, we use tools and concepts from the theory of complex networks to analyze resting state fMRI data of the brains of human subjects under two distinct conditions: (i) under ordinary waking state and (ii) in an altered state of consciousness induced by ingestion of Ayahuasca. We report an increase in the Shannon entropy of the degree distribution of the networks subsequent to Ayahuasca ingestion. We also find increased local and decreased global network integration. Our results are broadly consistent with the entropic brain hypothesis. Finally, we discuss our findings in the context of descriptions of "mind-expansion" frequently seen in self-reports of users of psychedelic drugs.

Relatively little is known about how exactly psychedelics act on human functional brain networks. During the last few years, new neuroimaging techniques, such as functional magnetic resonance imaging (fMRI)[1,2], have allowed noninvasive investigation of global brain activity in a variety of conditions, e.g., under anaesthesia, sleep, coma, and in altered states of consciousness induced by psychedelic drugs[3–10]. Recently, Carhart-Harris *et al.* proposed a hypothesis known as the *entropic brain*, which holds that the stylized facts concerning altered states of consciousness induced by psychedelics can be partially explained in terms of higher entropy of the brain's functional connectivity[11]. Although until recently the entropy of the brain had never been directly measured, the entropic brain hypothesis is empirically supported by several recent studies. For example, Sarasso *et al.* have reported complex spatiotemporal cortical activation pattern during anesthesia with ketamine, which can induce vivid experiences ("ketamine dreams")[12]. Similarly, Petri *et al.* found that after administration of the psychedelic psilocybin, the brain's functional patterns undergo a dramatic change characterized by the appearance of many transient low-stability structures[13]. Perhaps the most convincing evidence supporting the hypothesis thus far has come from the study undertaken by Tagliazucchi *et al.*[14], who reported a larger repertoire of brain dynamical states during the psychedelic experience with psilocybin. They inferred an increase in the entropy of the functional connectivity in some regions of the brain, by studying the temporal evolution (i.e., dynamics) of the connectivity graphs. Similarly, Lebedev *et al.*[15] and separately Schartner *et al.*[16] have very recently reported evidence of increases in specific measures of entropy, the former with LSD and the latter with LSD, psilocybin and ketamine. Here we directly measure increases in entropy associated with the functional connectivity of the whole brain under the influence of a psychedelic. Specifically, we analyze fMRI functional connectivity of human subjects before and after they ingest the psychoactive brew Ayahuasca and report an increase in the Shannon entropy. This is the first time that the entropy of the functional networks of the human brain has been directly measured in altered states of consciousness on a global scale, i.e. considering the entire brain.

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Ayahuasca is a beverage of Amazonian indigenous origin and has legal status in Brazil in religious and scientific settings[17]. It contains the powerful psychedelic *N*,*N*- dimethyltryptamine (DMT), together with harmala alkaloids that are known to be monoamine oxidase inhibitors (MAOIs). The beverage is typically obtained by decoction of two plants from the Amazonian flora: the bush *Psychotria viridis*, that contains DMT, and the liana *Banisteriopsis caapi*, that contains MAOIs[18]. DMT is usually rapidly metabolized by monoamine oxidase (MAO), but the presence of MAOI allows DMT to cross the blood-brain barrier and to exert its effects[19–24]. Similar to LSD, mescaline and psilocybin[19–24], Ayahuasca can cause profound changes of perception and cognition, with users reporting increase of awareness, flexible thoughts, insights, disintegration of the self, and attentiveness[19,25]. There is growing interest in Ayahuasca, partially due to recent findings showing that it may be effective in treating mental disorders, such as depression[26,27] and behavioral addiction[28,29]. Similar therapeutic potential has also been pointed out for other psychedelics[9,23,30–33].

For analysis, we use tools and concepts from the field of complex networks, a brief history of which follows. The application of graph theory to phase transitions and complex systems led to significant progress in understanding a variety of cooperative phenomena over a period of several decades. In the 1960s, the books by Harary, especially *Graph Theory and Theoretical Physics*[34], introduced readers to powerful mathematical techniques. The chapter by Kastelyn, still considered to be a classic, showed that difficult combinatorial problems of exact enumeration could be attacked via graph theory, including the exact solution of the two-dimensional Ising model (e.g., see Feynman[35]). In the 1980s, certain families of neural network models were shown to be equivalent to Ising systems, e.g., the Hopfield network[36] is a content-addressable memory which is isomorphic to a generalized Ising model[37]. Beginning in the 1990s, new approaches to networks, giving emphasis to concepts such as the node degree distribution, clustering, assortativity, small-worldliness; and network efficiencies, led eventually to what has become the new field of complex networks[38,39]. These new tools and concepts[40–42] have found successful application in the study of diverse phenomena, such as air transportation networks[43], terrorist networks[44], gene regulatory networks[45], and functional brain networks[46–50]. We approach the human brain from this perspective of complex networks[51,52].

Ten healthy volunteers were submitted to two distinct fMRI scanning sessions: (i) before and (ii) 40 minutes after Ayahuasca intake, when the subjective effects become noticeable (the volunteers drank 2.2 mL/kg of body weight and the Ayahuasca contained 0.8 mg/mL of DMT and 0.21 mg/mL of harmine, see Methods section). In both cases, participants were instructed to close their eyes and remain awake and at rest, without performing any task. See Methods for details concerning data acquisition and preprocessing.

Data analysis consists of two main steps. In the first step, we use fMRI data to generate complex networks to represent the actual functional brain connectivity patterns. In the second step, we use the networks generated in step 1 as inputs and calculate network characteristics as output, using techniques from the theory of complex networks. The Methods section describes both steps in detail. More information about most of the methods used here can be found in refs 4, 53, 54.

Figure 1 shows the networks generated from one subject before and after Ayahuasca intake, for one specific choice of mean node degree. The spheres represent nodes, with sphere size proportional to the degree of the node. The lower plots show histograms of node degrees.

The main result that we report here is an increase in the Shannon entropy of the degree distribution for the functional brain networks subsequent to Ayahuasca ingestion. We also find that the geodesic distance increases during the effects of Ayahuasca, i.e. qualitatively the network becomes "larger". More generally, we also find that these functional brain networks become less connected globally but more connected locally.

The key technical innovation is the measurement of the Shannon entropy of the degree distribution of the complex networks that represent the functional connectivity of the human brain. Moreover, the Shannon entropy is also very closely related to the Boltzmann-Gibbs entropy used in statistical mechanics. Hence, our approach to studying the brain experimentally is grounded in two strong theoretical traditions: graph theory and complex networks on the one hand, and information theory and statistical physics on the other. Our study also represents a significant advance for the following additional reasons: (i) our results unveil how Ayahuasca (and likely most other tryptamine psychedelics) alter brain function, both locally and globally; (ii) it is the first time this specific approach has been applied to characterize functional brain networks in altered states of consciousness; (iii) our study of Ayahuasca covers all brain regions; and (vi) the method we have developed can be immediately applied to study a variety of other phenomena (e.g., the effects of medication for mental health disorders).

Results

Increase of the Shannon entropy of the degree distributions. We find evidence of significant changes in the functional brain networks of subjects before and after ingestion of Ayahuasca. Figure 2 shows 2nd as well as 4th central moments of the degree distributions for each subject. The individual values are calculated separately for each network. We find an increase of variance for all subjects after Ayahuasca intake and a decrease of kurtosis for almost all of them (6 subjects). These findings indicate that the degree distributions become less peaked and wider. This behavior is suggestive of an increase of the Shannon entropy for the degree distributions after Ayahuasca ingestion.

Figure 3 shows the average Shannon entropy of the degree distributions as a function of mean degree, considering networks from all subjects, before and after Ayahuasca intake. A fair comparison of the "before" and "after" networks is possible by considering the entropy of networks of identical mean degree. We find an increase in the entropy of the degree distributions after Ayahuasca ingestion. In order to better evaluate the consistency of this result, we also calculate the average Shannon entropy subject-by-subject, before and after Ayahuasca (Fig. 4). We find significant increased entropy for all individual subjects.

To ensure that the observed changes in entropy are not due to augmented motion of subjects' heads during the effects of Ayahuasca, we also study the correlation between changes in individual Frame Displacement (FD)[55]

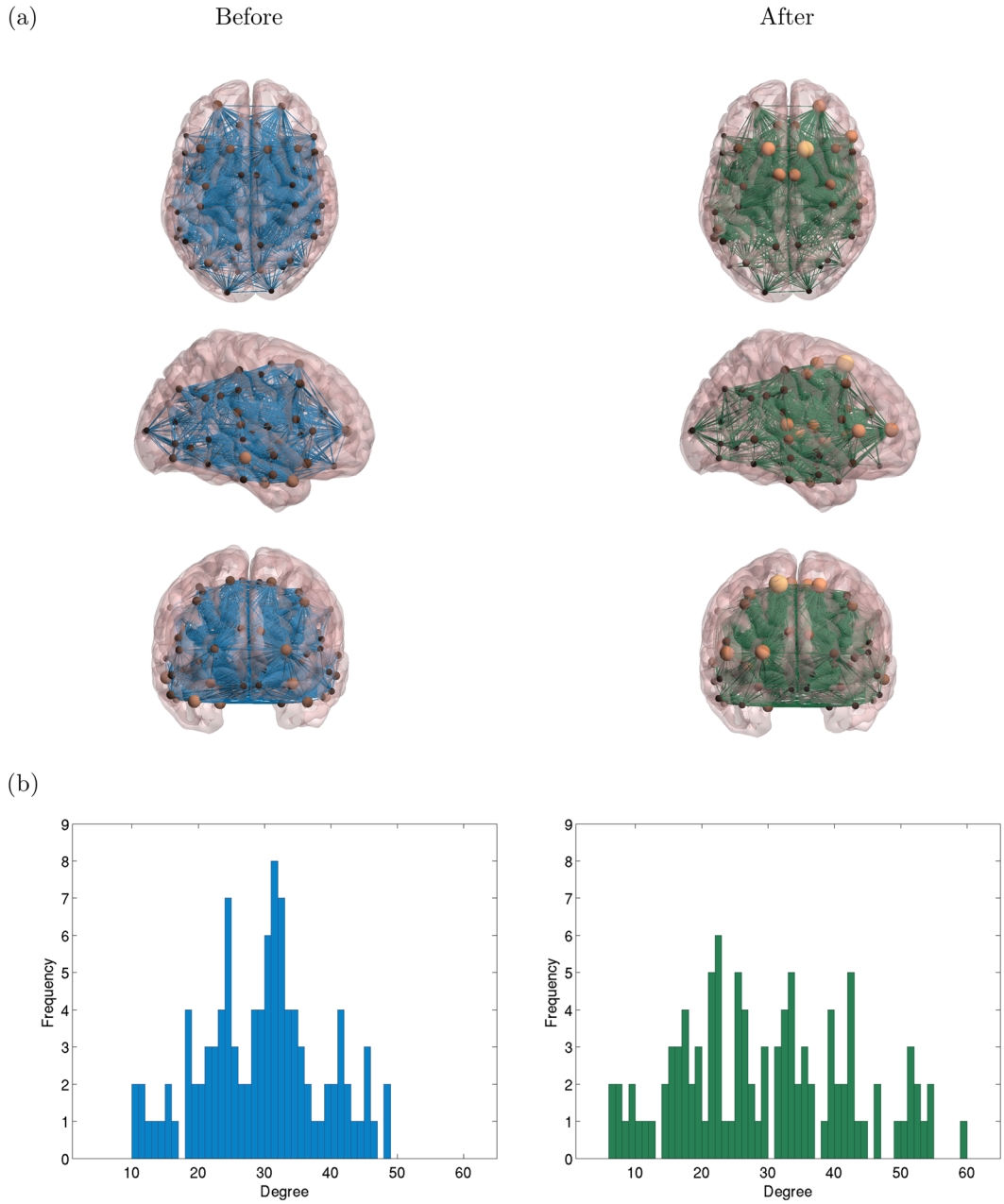

Figure 1. Illustrative example of functional brain networks. (**a**) 3 views of a complex network generated from fMRI data from one subject, before (left) and after (right) Ayahuasca ingestion (mean node degree $\langle k \rangle = 30$). The spheres represent nodes and sphere size is proportional to the node degree. (**b**) histograms of node degrees, corresponding to the networks shown in (**a**). After Ayahuasca intake, the distribution is wider, indicating a higher entropy. In (**a**) we have used the BrainNet Viewer (http://www.nitrc.org/projects/bnv) for visualization.

against individual changes in entropy. We find a Pearson correlation coefficient of 0.005 (hence, p-value ≈ 1), between entropy increases and motion (see Supplementary Figure S1). Hence, we discard the possibility that the observed entropy changes are due to augmented motion.

Iso-entropic randomized networks. The degree distribution does not completely define a network, however it can have great influence over other network properties. One can quantify this influence by comparing any given network G to other networks chosen randomly from the ensemble of networks that have exactly the same degree distribution. We refer to such networks as "randomized networks". By definition, all such randomized networks have the same entropy as the original network G, i.e. they are iso-entropic to G.

An efficient way of generating such randomized networks is the Maslov algorithm[56] (see Methods). Whereas entropy is conserved by the Maslov algorithm, the clustering coefficient, geodesic distances and efficiencies are not. By comparing these non-conserved quantities before and after randomization, we can distinguish effects that

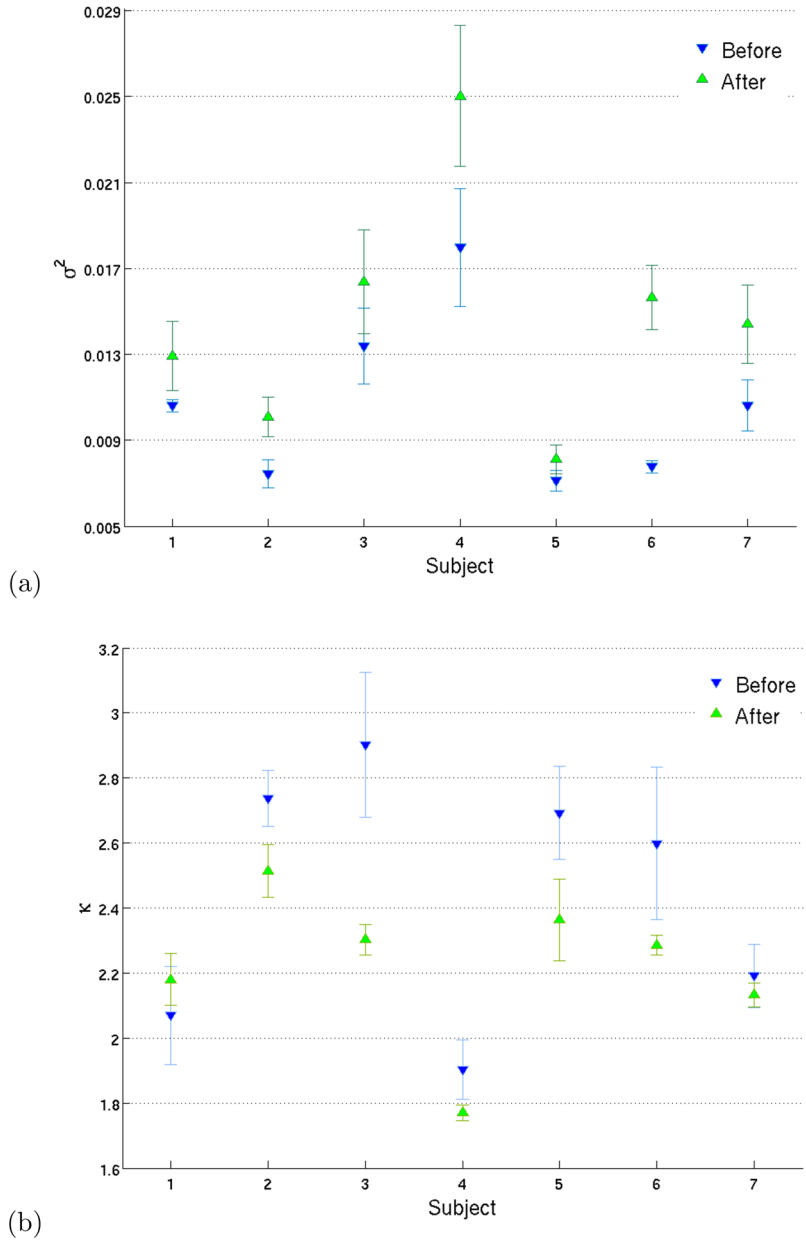

Figure 2. Variance and kurtosis of the degree distribution. Mean ± 1 standard deviation calculated over all 16 networks of the degree variance (**a**) and kurtosis (**b**), shown for each subject (blue ∇) and after (green Δ) Ayahuasca ingestion. The individual values for the degree variance and kurtosis are calculated separately for each network. We find higher variance and (mostly) lower kurtosis after Ayahuasca, hence the node distributions change shape and become less "peaked". Such behavior is again consistent with (if not suggestive of) a higher Shannon entropy after Ayahuasca.

are due solely to changes in the degree distribution from those that are sensitive to how links are more specifically arranged.

We generate a set of 30 iso-entropic randomized networks for each original network, for all subjects both before and after Ayahuasca ingestion. Comparison of the original networks with the randomized networks yields important information concerning to what degree the changes in quantities such as geodesic distance, clustering coefficients, and global and local efficiencies can be accounted for by the changes in the degree distributions (see results described below).

Decrease of global integration. Figure 5 shows an increase of mean geodesic distance and a decrease of global efficiency after Ayahuasca ingestion. To determine how much of the change in geodesic distance is due to the change in the degree distribution, we also calculated the geodesic distance and global efficiency for the iso-entropic randomized networks. Note how the values for those networks are quite different compared to the non-randomized networks. We conclude that the change in degree distribution cannot explain the entire change

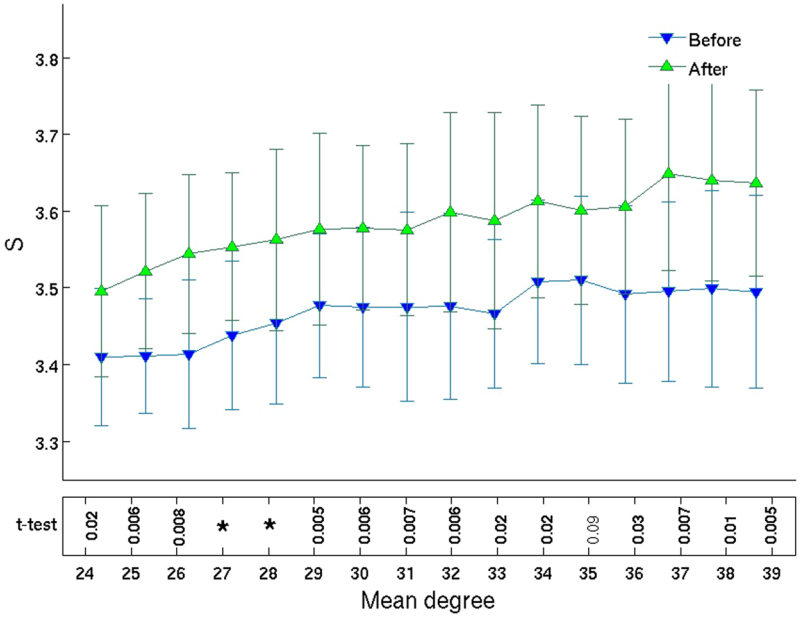

Figure 3. Entropy grows after Ayahuasca ingestion. Mean ± 1 standard deviation of the Shannon entropy of the distribution of node degrees, calculated over all 7 subjects, as a function of mean degree k, before (blue ∇) and after (green Δ) Ayahuasca intake. The bottom row lists p-values for Student's paired t-test, with values $p < 0.005$ indicated by asterisks (*). (Confidence intervals are shown in Supplementary Figure S5). We thus see evidence against the null hypothesis of no change in entropy. Indeed, we find a significant increase in the entropy of the degree distributions after Ayahuasca ingestion. This entropy increase is the main result that we report.

in geodesic distance. The inset in the middle panels shows the change in the normalized mean geodesic distance and global efficiency, which we define as the ratio D/D_{rand} and similarly for the global efficiency (see refs 4, 53). We see, indeed, that these ratios are not close to zero. If the change in degree distribution could account for all the change in geodesic distance and efficiency, then the change in these ratios would be close to zero. Significant changes are also observed at the individual level and are again consistent for all subjects (Fig. 5(e) and (f)).

Increase of local integration. Figure 6 shows an increase of clustering coefficients and local efficiency after Ayahuasca ingestion. In contrast to the behavior of the metrics discussed above, almost identical changes are seen for iso-entropic networks. This result indicates that the variation in degree distribution can account for most of the change in clustering and local efficiency. The insets in the middle panel show the change in the normalized clustering and local efficiency, which we define as the ratio C/C_{rand} and similarly for the local efficiency. We see, indeed, that these ratios are close to zero.

Subjective evaluation. The subjective states of the subjects were evaluated using two psychometric scales: the Clinician Administered Dissociative States Scale (CADSS)[57] and the Brief Psychiatric Rating Scale (BPRS)[58]. We report a significant correlation ($r = 0.91$; $p = 0.004$) between individual CADSS scores variation and the entropy before Ayahuasca intake (see Supplementary Fig. S2), however one must proceed with caution when interpreting these findigns because of the small number of subjects. The correlations between CADSS score variation and the variation in geodesic distance and global efficiency reach $r = 0.71$ and $r = 0.67$, respectively. Although these correlations fall short of our significance criterion ($r > 0.76$, $p < 0.05$), yet these r values are relatively large when compared with those for the clustering coeficient ($r = 0.15$) and local efficiency ($r = 0.09$).

Discussion

Our results reveal that the entropy increases after Ayahuasca ingestion. The following also increase: geodesic distance, clustering coefficient and local efficiency. However, the global efficiency decreases. Overall, we find an increase of local integration and a decrease of global integration in the functional brain networks.

We interpret these findings in the context of some well understood prototypical classes of networks. Regular lattices have fixed coordination number, hence all nodes have the same degree and the Shannon entropy of the degree distribution is thus zero. In contrast, the entropy is high in networks with broad distributions of degree. The observed increase in entropy after Ayahuasca ingestion indicates that the degree distribution becomes broader. In the context of the Watts-Strogatz model[59], clustering and geodesic distance both decrease when highly regular networks are transformed into small-world networks by randomly re-assigning the links. Whereas clustering and geodesic distances decrease with increasing randomness in such models, we find the opposite behavior for Ayahuasca, i.e., randomness as measured by the Shannon entropy of the node degree distribution increases in parallel with clustering and geodesic distances. Hence, our findings cannot be reduced to simple explanations of greater or lesser randomness. Locally, there is an increase in integration (as measured by network efficiency), but globally there is a decrease in integration. Indeed the increase of geodesic distance and decrease of global

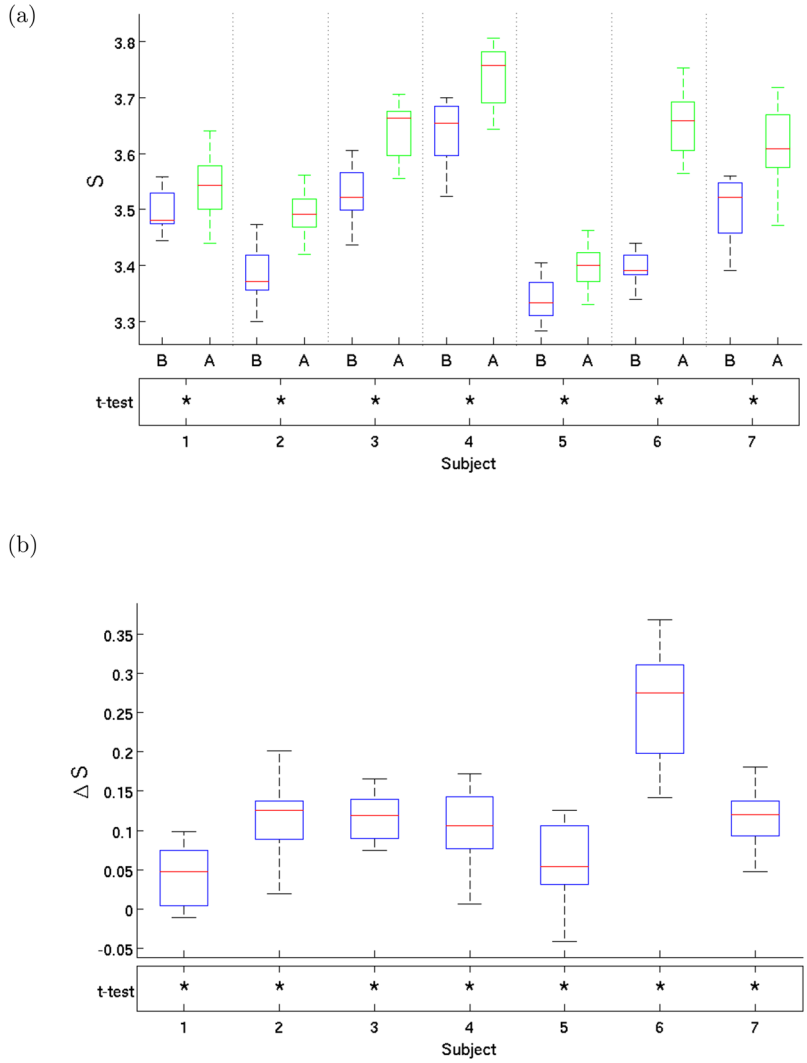

Figure 4. Entropy growth per subject. (**a**) Boxplot of the entropy distribution and before (**B**) and after (**A**) Ayahuasca ingestion and (**b**) boxplot of entropy increase, for all 7 subjects. Note the significant increase in entropy after Ayahuasca ingestion. There are 16 values of entropy per subject, as discussed in the text. The bars show minimum and maximum values and the box shows the 2nd and 3th quartiles, with the median shown dividing the box (in red). The asterisks (*) in the bottom rows in both plots indicate p-values $p < 0.005$ for Student's paired t-test in (**a**) and t-test for zero mean in (**b**). Subject-by-subject, we thus find strong evidence against the null hypothesis of no entropy change.

efficiency after Ayahuasca intake signify that the functional brain networks are less globally integrated. One possible interpretation of these findings is that the increase of local robustness and the decrease of global integration reflect a variation in modular structure of the network. Recent studies have reported the presence of modularity in functional brain networks on several scales[50, 60, 61]. Modular networks are characterized by the existence of reasonably well-defined subnetworks in which internal connections are denser than connections between distinct subnetworks[50]. However, traditional algorithms[62–64] were not able to detect variation on modular structure features between our sets of networks.

Our results are broadly consistent with the entropic brain hypothesis, hence we discuss the latter in the context of our findings. The hypothesis maintains that the mental state induced by psychedelics, which the original authors term "primary-state," presents relatively elevated entropy in some features of brain organization, compared to the ordinary waking state (termed "secondary")[11]. Although it may be somewhat counter-intuitive that the psychedelic state is considered primary while ordinary consciousness is secondary, their hypothesis is inherently plausible considering that a wider spectrum of experiences is possible with psychedelics than in ordinary consciousness. In this sense, ordinary consciousness can be thought of as a restriction or constrained special case of a more primary consciousness. The hypothesized lower entropy of ordinary consciousness relative to primary consciousness is attributed to this reduction of freedom. In fact, the idea that ordinary consciousness is not primary was previously put forth by Alan Watts to describe what later became widely known as *mindfulness*[65]. (We emphasize that the terms "primary" and "secondary" are used not as value judgements, but rather to

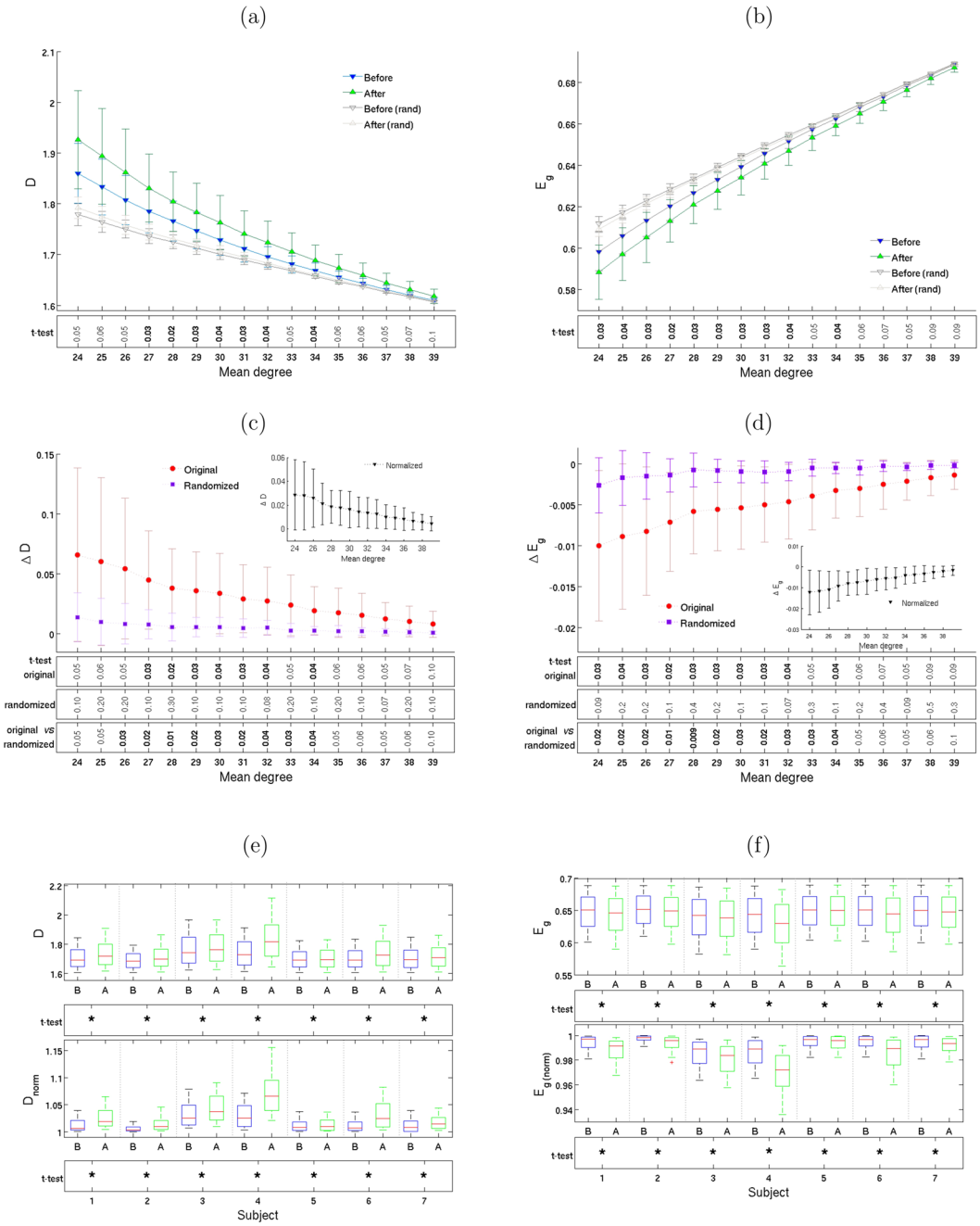

Figure 5. Global efficiency and integration decrease. Geodesic distance D (left column) and global efficiency E_g (right column). Plots (**a**) and (**b**) show means ± 1 standard deviations, calculated from the complex networks of all 7 subjects, as well as from their corresponding iso-entropic randomized networks, for 16 different mean degrees. Plots (**c**) and (**d**) show the change in D and E_g after Ayahuasca ingestion. The inset shows normalized values (see text). Boxplots (**e**) and (**f**) show the same information, subject-by-subject. As in previous figures, the rows below the plots show p-values for the t-test, with asterisks (*) indicating $p < 0.005$.

denote temporal order, i.e. the order of appearance, both evolutionarily as well as in the maturation of a healthy individual).

More specifically, we believe that the observed increase in entropy may be related to the temporary removal of the some of the restrictions that are necessary for sustaining ordinary (adult trained) consciousness. With these restrictions temporarily gone, the entropy increases and the mind may become effectively more "free", attaining a more flexible state in which self-referential narratives and thoughts about the past or the future are no longer mentally identified with the reality that they represent. We emphasize, however, that "correlation is not causation" and that the entropy increases may occurr in parallel with the loss of constraints, rather than bear a direct causal relation to them. These ideas are further explored in the Supplementary Discussion (see also refs. [80–83]).

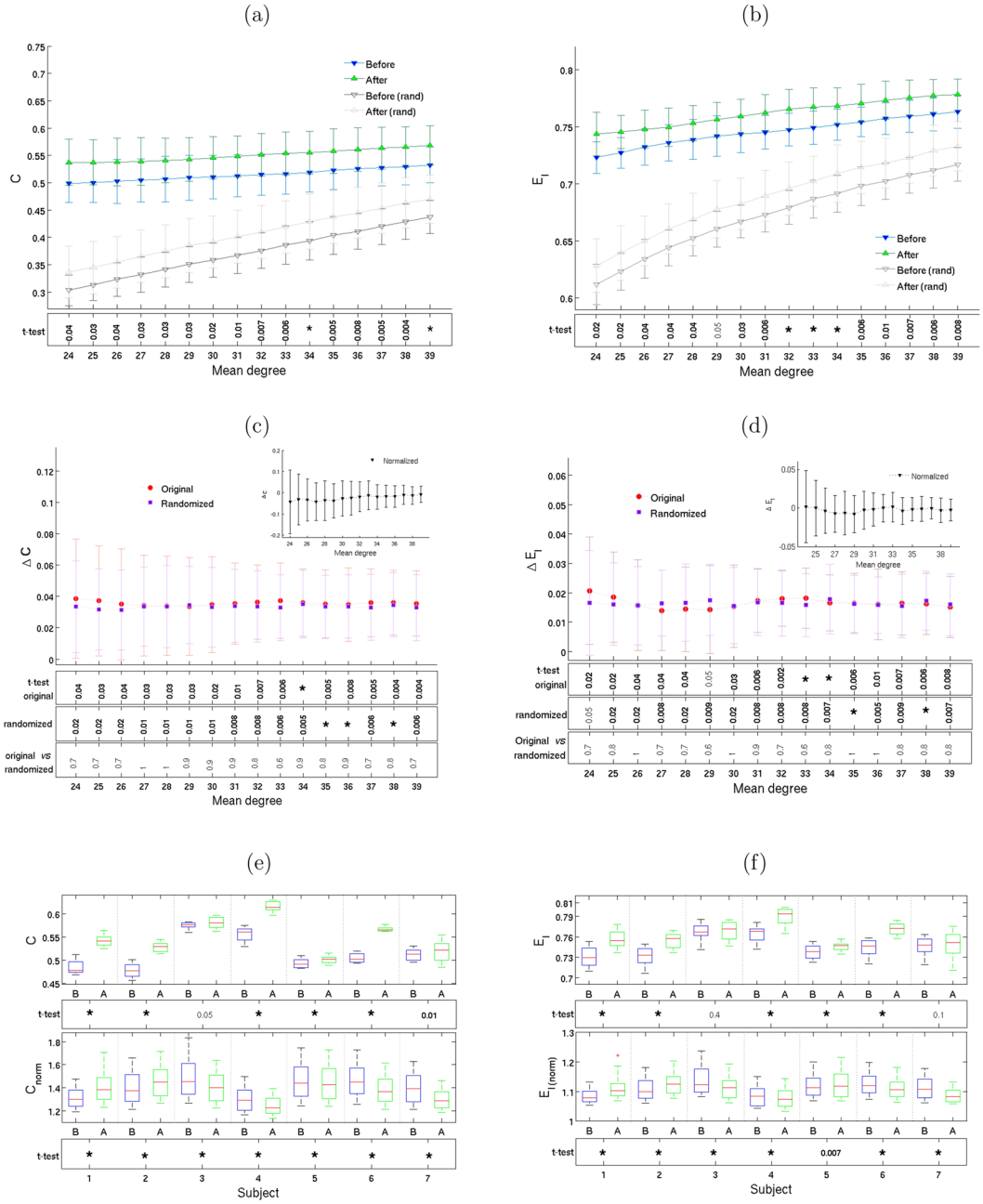

Figure 6. Local efficiency and integration increase. Clustering coefficient C (right column) and local efficiency E_l (left column). Plots (**a**) and (**b**) show means ± 1 standard deviations, calculated from the complex networks of all 7 subjects, as well as from their corresponding iso-entropic randomized networks, for 16 different mean degrees. Plots (**c**) and (**d**) show the change in C and E_l after Ayahuasca ingestion. The inset shows normalized values (see text). Boxplots (**e**) and (**f**) show the same information, subject-by-subject. As before, the rows below the plots show p-values for the t-test, with asterisks (*) indicating $p < 0.005$.

Relatively few studies have investigated entropy in brain functional networks, hence additional comments are in order. Tagliazucchi *et al.*[14] showed that psilocybin (psychedelic present in some species of mushrooms) may be responsible for increases of a different entropy measure in functional connectivity of the 4 regions of Default Mode Network (DMN), a relevant functional network related to resting state. Recently, Yao *et al.*[66] correlated entropy increases in the human brain with age. This study also supports the view that entropy is correlated to brain function (and perhaps also its development). Moreover, in agreement with our results, a study by Schröter *et al.*[4] similarly suggests that functional network topology may have a central role in consciousness quality. They investigated the effects on the human brain of the anesthetic propofol, which can induce loss of consciousness[67]. They reported a decrease of the clustering coefficient, which is strongly influenced by degree distribution (however, geodesic distance remained unchanged). Very recently, Lebedev *et al.*[15] and separately Schartner *et al.*[16] have

reported evidence of increases in measures of entropy, the former with LSD and the latter with LSD, psilocybin and ketamine.

We briefly comment on the limitations of our method: (i) the reduced number of subjects and the fact that all of them were experienced with Ayahuasca do not allow population inferences and do not elucidate whether the effects observed here were only due the acute administration or if previous experience also played a significant role; (ii) expectancy and suggestion were not controlled, as placebo was not used; (iii) networks were built upon a number of critical choices, such as the atlas used to partition the brain, the method used to build the correlation matrix, and the cutoff thresholds for generating the adjacency matrices from correlation matrices[68,69], which may affect the final results; (iv) the chosen range of correlation values automatically limits the networks' behavior to a small-world network. Despite this limitation, it is important to highlight that several studies have consistently demonstrated that brain networks exhibit a small-world behavior[70].

Finally, we speculate about whether or not our finding of larger mean geodesic distances may have any relation to self-reports of "mind-expansion" by users of psychedelics (see also ref. 16). Could there be a direct relation between entropy increases and the higher creativity reported by users of psychedelics? Such questions merit further investigation. In conclusion, our results are broadly consistent with the hypothesis that psychedelics increase the entropy in brain functions. By calculating the Shannon entropy of the degree distribution of complex networks generated from fMRI data, we have taken a new low-computational-cost approach to investigating brain function under the influence of psychedelics.

Methods

Data acquisition and preprocessing. The fMRI images were obtained in a 1.5 T scanner (Siemens, Magneton Vision), using an EPI-BOLD like sequence comprising 150 volumes, with the following parameters: TR = 1700 ms; TE = 66 ms; FOV = 220 mm; matrix 64 × 64; voxel dimensions of 1.72 mm × 1.72 mm × 1.72 mm. It also was acquired whole brain high resolution T1-weighted images (156 contiguous sagittal slices) using a multiplanar reconstructed gradient-echo sequence, with the following parameters: TR = 9.7 ms; TE = 44 ms; flip angle 12°; matrix 256 × 256; FOV = 256 mm, voxel size = 1 *mm* × 1 *mm* × 1 *mm*. The images were obtained from 10 healthy right-handed adult volunteers (mean age 31.3, from 24 to 47 years), all who were experienced users of Ayahuasca with at least 5 years use (twice a month) and at least 8 years of formal education. The experimental procedure was approved by the Ethics and Research Committee of the University of São Paulo at Ribeirão Preto (process number 14672/2006). Written informed consent was obtained from all volunteers, who belonged to the Santo Daime religious organization. All experimental procedures were performed in accordance with the relevant guidelines and regulations.

Volunteers were not under medication for at least 3 months prior to the scanning session and were abstinent from caffeine, nicotine and alcohol prior to the acquisition. They had no history of neurological or psychiatric disorders, as assessed by DSM-IV structured interview[71]. Subjects ingested 120–200 mL (2.2 mL/kg of body weight) of Ayahuasca known to contain 0.8 mg/mL of DMT and 0.21 mg/mL of harmine. Harmaline was not detected via the chromatography analysis, at the threshold of 0.02 mg/mL[7]. Preprocessing steps were conducted in FSL (http://www.ndcn.ox.ac.uk/divisions/fmrib) and include: slice-timing correction, head motion correction and spatial smoothing (Gaussian kernel, FWHM = 5 mm). One volunteer was excluded from analysis due to excessive head movement (more than 3 mm in some direction), leaving 9 participants (5 women) to our analysis. All images were spatially normalized to the Montreal Neurologic Institute (MNI152)[72] standard space, using a linear transformation. We also evaluated 9 regressors of non-interest using a General Linear Model (GLM): 6 regressors to movement correction, 1 to white matter signal, 1 to cerebrospinal fluid and 1 to global signal. Each volunteer was submitted to fMRI scanning under two distinct conditions: (i) before and (ii) 40 minutes subsequent to Ayahuasca intake. In both cases, volunteers were in an awake resting state: they were requested to stay lying with eyes closed, without performing any task.

Complex network metrics. For a detailed overview of complex network theory, we refer readers to refs 38, 41, 49. Each element of a network is known as a node (or vertex), and the relation between a pair of nodes is represented by a connecting link (or edge). Links can have weights associated with them and can be directed or undirected (or, equivalently bi-directional). Nodes connected by a single link are known as nearest neighbors[39]. Non-weighted undirected networks, i.e. those with symmetric and unweighted links are isomorphic to a binary symmetric matrix known as the adjacency matrix. When a pair of nodes i and j are neighbors, the adjacency matrix element is $a_{i,j} = 1$ and $a_{i,j} = 0$ otherwise[73,74]. Standard quantities of interest that help to characterize the topology and complexity of networks[49,53] include node degree, geodesic distance, clustering coefficient, and local and global network efficiencies. We are interested here solely in non-weighted undirected networks.

Definitions:

(i) The degree k_j of a node j is the number of links that it has with other nodes. The degree distribution of a network is the normalized histogram of degrees over all nodes.

(ii) A geodesic path between two nodes is the shortest path from one to the other, assuming such a path exists. The geodesic distance $d_{i,j}$ between nodes i and j is the number of links in the geodesic path. If there is no such path, the geodesic distance is defined as infinite. Given a network G with N nodes, the mean geodesic distance is given by

$$D(G) = \frac{1}{N(N-1)} \sum_{i \neq j} d_{i,j}. \tag{1}$$

(iii) The clustering coefficient quantifies the density of triads of linked nodes, e.g., the fraction of the neighbors

of a node that are themselves neighbors. The clustering coefficient is defined by

$$C(G) = \frac{1}{N}\sum_{i\neq j\neq h}\frac{2}{k_i(k_i-1)}a_{i,j}a_{j,h}a_{h,i}, \tag{2}$$

where k_i is the degree of node i and a is the adjacency matrix element.

(iv) The efficiency, typically defined as the reciprocal of the harmonic mean of geodesic distances, quantifies the influence of the topology on flux of information through the network. Efficiency can be global as well as local. We define global efficiency as

$$E_g(G) = \frac{1}{N(N-1)}\sum_{i\neq j\in G}\frac{1}{d_{i,j}}, \tag{3}$$

and local efficiency as

$$E_l(G) = \frac{1}{N}\sum_{i\in G}\left(\frac{1}{n_i(n_i-1)}\sum_{j\neq h\in g_i}\frac{1}{d_{h,j}}\right), \tag{4}$$

where g_i are the subnetworks formed by neighbors of node i and n_i is the number of nodes of this subnetwork[75].

In addition to these standard network properties, we also use the Shannon entropy[76] to quantify disorder or uncertainty. Specifically, we calculate the Shannon entropy functional of the distribution of node degrees. Let P be the normalized probability distribution for node degree k, i.e. $\sum_k P(k) = 1$. We define the Shannon entropy $S[P]$ of the degree distribution $P(k)$ for a network with N nodes by:

$$S[P] = -\sum_k P(k)\log P(k). \tag{5}$$

Often the logarithm of base 2 is used[77] (e.g., in computer science), but we use the natural logarithm instead, so the entropy values shown are in natural information units rather than in bits.

Maslov algorithm for generating randomized networks. Given G, one can select two non-overlapping pairs (i, j) and (m, n) of linked nodes, then unlink them, and cross-link the pairs according to (i, m) and (j, n). If this process is repeated many times, the links become randomized, but the degree of each node remains the same[56]. Hence the entropy of the degree distribution is also a conserved quantity.

Calculation of correlation matrix for brain regions. We segmented the brain images into 110 brain regions according to the Harvard-Oxford cortical and subcortical structural atlas (threshold of >25%, using FMRIB Software Library, www.fmrib.ox.ac.uk/fsl). Six regions had to be excluded from further analysis, as they were not sampled for all subjects, due to technical limitations during image acquisition. For each of the 104 regions, an averaged fMRI time series was computed from all voxels (a voxel is a 3D image block, analogous to the 2D pixel). within that region using Marsbar (SPM toolbox). To reduce confounders, we applied a band-pass filter (≈0.03–0.07 Hz) using the maximum overlap wavelet transform (MODWT) with a Daubechies wavelet to divide the signal into 4 scales of different frequency bands. In keeping with the literature[4,54], that point that resting state typically leads to low frequency (≈0.01 to 0.1 Hz)[78], we choose scale 3. We then calculated the Pearson correlation between these wavelet coefficients from all possible pairs, thus obtaining a 104×104 correlation matrix to represent each sample. We considered only correlation matrix elements that resulted in $p < 0.05$ in the hypothesis test. That is, we zero correlation matrix elements with non-reliable values ($p \geq 0.05$).

Construction of complex networks from fMRI images. A correlation matrix uniquely may define a weighted network. Nonetheless, we are interested in generating non-weighted networks. Hence, we need a function that maps correlation matrices to adjacency matrices. We use a thresholding function for this purpose. Given a correlation matrix, we obtain the adjacency matrix by applying a threshold to the absolute value of the elements of the correlation matrix. Specifically, if the absolute value of the correlation matrix element $|c_{i,j}|$ is larger than a defined threshold η, then a link is assumed and the adjacency matrix element is taken to be 1 (i.e., $a_{i,j} = 1$), while otherwise there is no link ($a_{i,j} = 0$). In order to obtain better statistics, we choose not a single value of η but a range of values instead. Then we analyze the behavior of the network properties over this range. Using this approach, we create a number of networks for each fMRI sample, all with the same number of nodes (104 nodes). For each of these networks, we choose η such that the density of links is the same before and after Ayahuasca intake.

We choose a range for the mean network degree to ensure the networks were fully connected but also relatively sparse. For this purpose, we adopt the following criteria: (i) the lowest correlation threshold (corresponding to maximum mean degree) must ensure that the networks have lower global efficiency and greater local efficiency than its randomized version. These criteria also ensure small-world behavior of the networks[79] (according to the definition of Watts and Strogatz[59]). (ii) the upper correlation threshold (minimum mean degree) must ensure fully connected graphs and also guarantee that the networks obey the sparsity condition $\langle k\rangle > 2\ln N$, where $N = 104$ is the number of nodes. Since the quantity $2\ln N$ never exceeds 10, our maximum threshold condition (minimum mean degree) was determined by the requirement for obtaining fully connected graphs. (A properly defined large N limit of this condition would be equivalent to the percolation threshold).

We choose common upper and lower thresholds for all correlation matrices (see Supplementary Fig. S3 for more details). These criteria are identical to those adopted by refs 4, 54. In order to obtain the same threshold

range for all subjects, it was necessary to exclude two of them from the analysis, since there is no threshold range common between them and the other subjects. Data from a third subject was also excluded due to excessive head movement. Following the criteria described above, the threshold range is set to $0.25 \leq \eta \leq 0.43$. We generate networks with mean degree in the range $24 \leq \langle k \rangle \leq 39$. We have shown in Figs 3, 5 and 6 network properties as a function of mean degree in increments of $\Delta\langle k \rangle = 1$, for 16 different values of mean degree. We choose mean degree as the independent parameter instead of the correlation threshold because, unlike the latter, the mean degree is a network property. Despite the mean degree being a monotonic function of correlation threshold for all subjects, there is no *a priori* reason to expect universal behavior of network properties such as the entropy and geodesic distance (before vs. after Ayahuasca) as a function of the correlation threshold since the latter is not a network property (see Supplementary Fig. S3).

In summary, we have 7 human subjects suitable for both conditions (before and after ingestion). The resulting sets of networks allow 16 different comparisons (i.e. of differing mean degrees) for each subject before and after Ayahuasca ingestion. We calculate the topological measurements (using the Brain Connectivity Toolbox for Matlab[49]).

Statistical testing. Comparisons between the two conditions (i.e., before and after Ayahuasca) are obtained from paired-sample Student's *t*-tests. The *p*-values shown in Figs 3, 4, 5 and 6 are as follows: values $p < 0.05$ in bold and $p < 0.005$ indicated by asterisks (*). The implicitly assumed null hypothesis is that the difference of the paired values are normally distributed with zero mean.

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Acknowledgements
We thank Santo Daime members for volunteering and for providing the Ayahuasca. We thank Sidarta Ribeiro for discussions, José C. Cressoni, Marcos G. E. da Luz, Ernesto P. Raposo, Marco A. A. da Silva, and Carlos Viol Barbosa for feedback and CAPES and CNPq for funding. AV thanks UFV and Science without Borders (CAPES Grant No. 88881.030375/2013-01) for funding and Guillermo Cecchi and Irina Rish for their hospitality and discussions during her year at IBM.

Author Contributions
D.B.A. recruited the volunteers for data acquisition and conceived the study. A.V., F.P.-F. and H.O. performed fMRI data preprocessing, complex network construction and evaluated standard network features. A.V. and G.M.V. performed complex network analysis, statistical analysis and wrote the first manuscript draft. D.B.A., F.P.-F. and H.O., reviewed the manuscript with input from the others. A.V. prepared all figures. All authors contributed equally to the final overall design of the study.

Additional Information
Supplementary information accompanies this paper at doi:10.1038/s41598-017-06854-0

Competing Interests: The authors declare that they have no competing interests.

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APÊNDICE C – Artigo publicado durante o doutorado

Epilepsy & Behavior 38 (2014) 71–80

Contents lists available at ScienceDirect

Epilepsy & Behavior

journal homepage: www.elsevier.com/locate/yebeh

Review

Brain complex network analysis by means of resting state fMRI and graph analysis: Will it be helpful in clinical epilepsy?

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ARTICLE INFO

Article history:
Revised 11 October 2013
Accepted 16 November 2013
Available online 27 December 2013

Keywords:
Complex network
Graph
fMRI
Resting state
Epilepsy

ABSTRACT

Functional magnetic resonance imaging (fMRI) has just completed 20 years of existence. It currently serves as a research tool in a broad range of human brain studies in normal and pathological conditions, as is the case of epilepsy. To date, most fMRI studies aimed at characterizing brain activity in response to various active paradigms. More recently, a number of strategies have been used to characterize the low-frequency oscillations of the ongoing fMRI signals when individuals are at rest. These datasets have been largely analyzed in the context of functional connectivity, which inspects the covariance of fMRI signals from different areas of the brain. In addition, resting state fMRI is progressively being used to evaluate complex network features of the brain. These strategies have been applied to a number of different problems in neuroscience, which include diseases such as Alzheimer's, schizophrenia, and epilepsy. Hence, we herein aimed at introducing the subject of complex network and how to use it for the analysis of fMRI data. This appears to be a promising strategy to be used in clinical epilepsy. Therefore, we also review the recent literature that has applied these ideas to the analysis of fMRI data in patients with epilepsy.

This article is part of a Special Issue entitled "NEWroscience 2013".

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1. Introduction

Complex systems consist of a large number of elements that mutually interact and that exhibit global behaviors or emergent proprieties not deducible from a simple local analysis. Such systems are not guided by central control, but rather they present self-organizing collective dynamics [1]. The notion of complex networks emerges in this context. Any complex system described mathematically by graph theory can be called a complex network [2]. In recent years, complex networks have become of major interest in the biological, technological, and social sciences, such as ecological networks, science of collaboration networks, the World Wide Web, social networks, and neuroscience [3], just to mention a few.

On the other hand, the advent of functional neuroimaging tools opened an important window for the noninvasive investigation of the human brain, among which functional magnetic resonance imaging (fMRI) plays a prominent role. It is capable of evaluating different brain sites over time, which is the basic need for the use of complex network strategies. In fact, the union of these two techniques has led to interesting results from basic [4,5] to clinical neuroscience [6–8].

Considering the growing number of studies in this area, we herein aim at providing an introduction to complex networks and a guideline of its application for the analysis of functional connectivity fMRI data. Furthermore, we review the recent literature that has applied these ideas to the analysis of fMRI data in patients with epilepsy.

2. Functional magnetic resonance imaging

Functional magnetic resonance imaging is based on the observation that the increase in neuronal electrical activity in a particular site is accompanied by a local increase in cerebral blood flow at the arteriole level [9]. This leads to a slight modulation of the MRI signal that gives rise to the contrast mechanism known as BOLD (blood oxygenation level-dependent) [10].

Typical fMRI experiments involve the serial acquisition of MRI scans with BOLD contrast images of the whole brain being collected every 1–2 s (depending on the repetition time —TR). Because of the low amplitude of such evoked signals (typically on the order of 3%), usually the subjects are asked to perform the same task several times, and a sophisticated strategy of analysis is employed to accurately detect statistically significant activated brain areas [11–17].

This technique has just completed 20 years [18]. It has been a reference tool in a broad range of human brain studies including higher

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http://dx.doi.org/10.1016/j.yebeh.2013.11.019

cognitive processes [19], pharmacological challenges [20,21], and clinical applications [22], as in the case of epilepsy [23].

Currently, fMRI plays an important role in assisting surgical treatment of patients with epilepsy [23]. Traditionally, it involves noninvasive functional preoperative mapping of eloquent areas such as the ones related to motor, language, and memory systems [23–25], as well as determining lateralization of receptive (Wernicke's area) and expressive (Broca's area) language fields [26]. However, these approaches are not of much help when it comes to the precise spatial localization of the epileptic activity, which is a central issue in the diagnosis and treatment of epilepsy. More recently, however, resting state fMRI has appeared as a promising approach.

Resting state fMRI focuses on spontaneous fluctuations of the BOLD signal, or the "intrinsic activity" of the brain, instead of looking at evoked activity. The term "intrinsic activity" summarizes any ongoing neural and metabolic activity that is not directly correlated to a specific task. It is usually recorded with the subject lying down relaxed with their eyes closed, without falling asleep, while continuous fMRI data are being collected [27].

Nowadays, several studies are using this approach to examine the existence and extent of intrinsic functional connections between brain regions, known as functional connectivity fMRI (fc-fMRI). These methods can be divided into two main groups: model-dependent or model-free. In the model-dependent method, regions of interest (seeds) are chosen based on a priori knowledge/question, from which time series are extracted and processed, aiming at evaluating if this region is functionally connected to other brain areas [28,29]. In the time domain, two mathematical approaches are frequently used: cross-correlation analysis (Pearson or partial correlation) and statistical parametric mapping [30–32]. One of the main disadvantages of these two approaches is that both are sensitive to the shape of the hemodynamic response, which is known to vary among different individuals and brain regions and with age [33–35]. Moreover, contamination from physiological processes, such as respiratory and cardiac oscillations, can lead to false high correlations [36,37]. To overcome these problems, one can use coherence, which is the representation of correlation in the frequency domain, to create functional maps that define functional connectivity of predefined brain regions. Although these maps provide information about the functional connectivity of the seeds, they cannot be used to study functional connectivity at a whole-brain scale. For this purpose, model-free methods can be used, as independent component analysis (ICA), principal component analysis (PCA), and clustering [28,38,39].

Besides functional connectivity, effective connectivity, the causal influence that a neural unit exerts over another, has also been the focus of attention. Two main techniques have been used: Granger causal modeling (GCM) and dynamic causal modeling (DCM). In the GCM, it is assumed that if one time series is a time-shifted version of the other, then the one with temporal precedence caused the other, giving a measure of directionality [27,40]. On the other hand, DCM is a hypothesis-led approach to study observed brain responses, in which hypotheses are outlined in a reasonably realistic neuronal model of interacting cortical regions (networks), and a Bayesian model is used to quantify the influence of one network over the other [41].

In addition to evaluating differences of functional or effective connectivity between pairs of brain regions, resting state fMRI is being progressively used to better understand how brain networks interact, using the framework of complex networks.

3. Brain complex networks

Although the study of complex networks is mathematically based on graph theory [42], the study of large-scale complex systems led to the development (and definitions) of new metrics and statistical observables. It is worth noting that the nomenclature may change according to the field of application, and, frequently, the terms "graph" and "network" are used synonymously. We have chosen to adopt, in what follows, the term "network" for all concepts related to networks and graphs. In this section, we introduce the basic terminology and quantities important in the science of complex networks.

A network is a way to code a set of elements together with their relations. The elements are defined as nodes (or vertices), and their

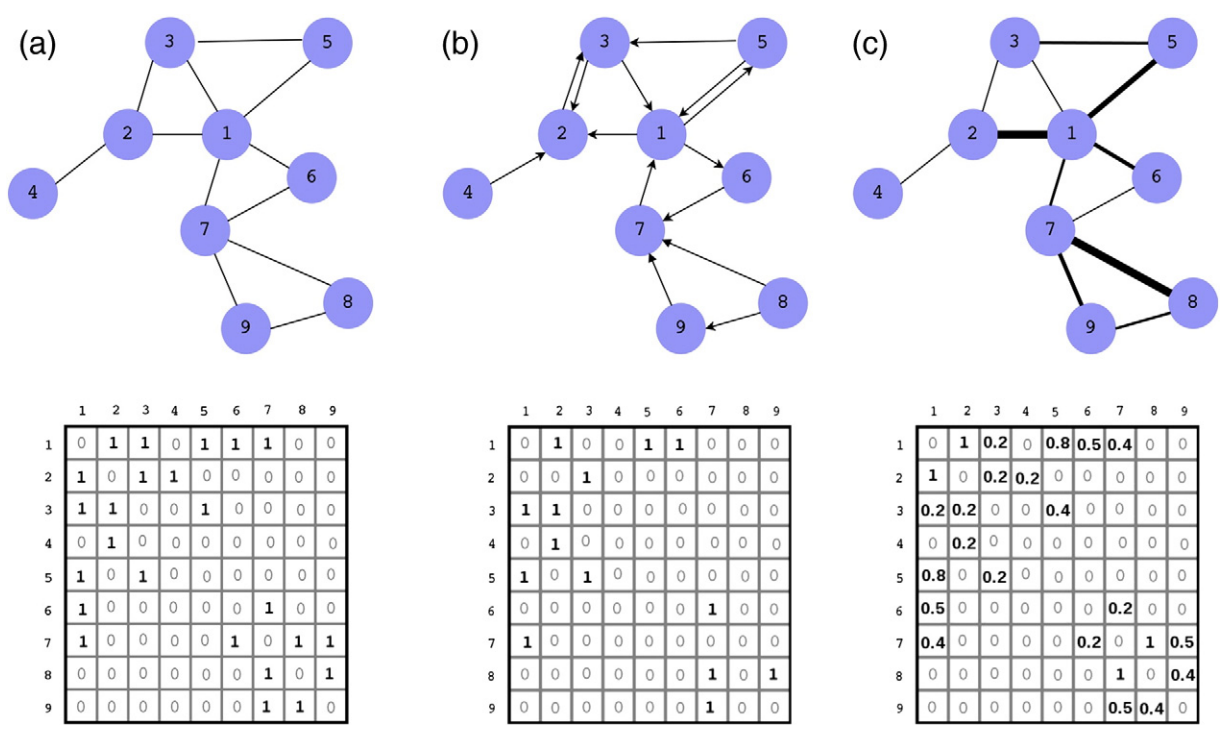


Fig. 1. Types of networks. Examples of undirected (a), directed (b), and weighted (c) networks and their corresponding adjacency matrices. See Refs. [2,49] for a more detailed introduction on complex networks.

relations are defined as links (or edges) [2]. For example, if nodes represent people in a social network, then links can represent friendships between pairs of people. Mathematically, a network is represented by ordered pairs of set $G(N,l)$ in which N is a set of nodes and l is a set of links. Graphically, the nodes are plotted as points and the links as lines joining them [2] (Fig. 1).

When two nodes are connected by a link, they are considered neighbors (or adjacent). A network with N nodes is said to have order N. A network structure that assigns a label (weight) to each link is called a weighted network. Otherwise, if the links of a network do not have labels, the network is called binary (or unweighted).

A common and very useful representation of a network is the adjacency matrix (or connection matrix), which completely defines the topology of the network. The matrix element $a(i,j)$ is nonzero if there is a link between nodes i and j. In binary adjacent matrices, the link between nodes i and j is represented by 1 and no connection by 0 (Fig. 1a). When the adjacency matrix is weighted and normalized, the value of $a(i,j)$ will lie in the interval from 0 to 1 (Fig. 1c). Moreover, network links can be classified as undirected or directed (Fig. 1b). In an undirected network, the number of links in a node, k, is the degree of this node. In directed network, the in-degree and the out-degree correspond to the number of in-coming and out-coming links, respectively. In this case, the sum of in-degree and out-degree is the degree of a node, k (Fig. 1b). The average degree of all nodes is the degree of network. Since most of the studies in brain networks have been carried out with binary undirected networks, we will focus on this class of networks.

3.1. Degree distribution

The degree distribution $P(k)$ is the normalized frequency histogram of the node degrees for the network (see Eq. (2) in Table 1), such that $P(k)$ is the probability that a randomly chosen node has degree equal to k [42] (Fig. 2). This definition is important because it contains information about network architecture and can be crucial for identifying the type of network [42]. For example, a heavy-tailed distribution

Table 1
Complex network measures: Equations and definitions.

Measure	Equation		Definitions
Degree of node [7]	$k_i = \sum_{j \in G} a(i,j)$	(1)	G represents the full set of network. $a(i,j)$ is the element of adjacency matrix. $a(i,j) = 1$ when there is a link between nodes i and j. $a(i,j) = 0$ otherwise.
Degree distribution [2]	$P(k) = \frac{n_k}{N}$	(2)	n_k is the total number of nodes with degree k. N is the total number of nodes.
Cost or probability of connection [7]	$P_{\text{cost}}(G) = \frac{1}{N(N-1)} \sum_{i \in G} k_i$	(3)	This metric is evaluated in the full network. G represents the network.
Cluster coefficient [47]	$C(G) = \frac{1}{N} \sum_{i \in V} C(i) = \frac{1}{N} \sum_{(i,j,h) \in G} \frac{2a(i,j)a(i,h)a(j,h)}{k_i(k_i-1)}$	(4)	$C(i)$ is the cluster coefficient of node i. $C(i) = 0$ for $k_i < 3$.
Transitivity [47]	$T(G) = \frac{\sum_{i \in G} 2a(i,j)a(i,h)a(j,h)}{\sum_{i \in G} k_i(k_i-1)}$	(5)	Unlike cluster coefficient, this metric is defined only to a full network.
Local efficiency [7]	$\mathrm{E}_{\text{local}}(i) = \frac{1}{N_{G_i}\left(N_{G_i}-1\right)} \sum_{j,k \in G_i} \frac{1}{d(j,k)}$	(6)	G_i is a set of neighbors of i.
Shortest path length [47]	$L = \frac{1}{N(N-1)} \sum_{i,j \in G, i \neq j} d(i,j)$	(7)	Where $d(i,j)$ is the shortest path length between i and j.
Global efficiency [7]	$\mathrm{E}_{\text{global}}(G) = \frac{1}{N(N-1)} \sum_{i \neq j \in G} \frac{1}{d(i,j)}$	(8)	$\mathrm{E}_{\text{global}}$ is evaluated in the full network.
"Small-worldness" [53]	$\sigma = \frac{\mathrm{C}/\mathrm{C}_{\text{rand}}}{\mathrm{L}/\mathrm{L}_{\text{rand}}}$	(9)	C_{rand} and L_{rand} are cluster coefficient and shortest path length evaluated to randomly network from original network. The network is small-world if $\sigma \gg 1$

The equations in this table refer to binary undirected networks.

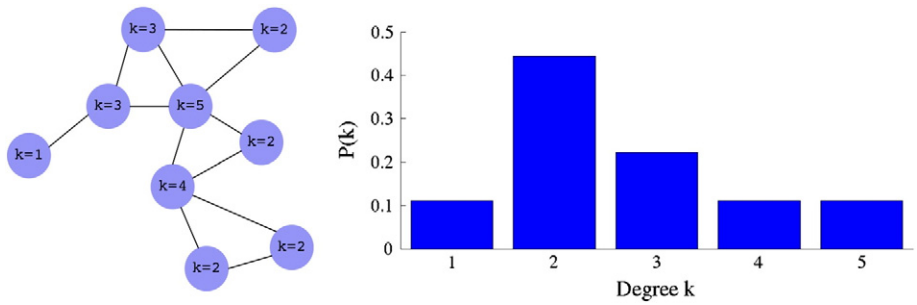


Fig. 2. Degree distribution. Illustrative example showing the degree of each node and, on the right, the degree distribution of the network.

indicates the existence of highly connected nodes, known as hubs, and their presence influences its information exchange efficiency and the network resilience, i.e., if a node is removed, the structure of the network will not change much qualitatively [43].

3.2. The cluster coefficient

The distribution of information processing in brain networks can be understood through the concept of functional segregation, and a possible way to infer this property is by quantifying how clustered the network is.

The concept of cluster is quite simple. If the nearest neighbors of a node are also directly connected to each other, they form a cluster. In the example of social networks, the friend of your friend is likely also your friend [44]. The clustering coefficient of a node, $C(i)$, is the ratio of the number of links that exist between the nearest neighbors of the chosen node and the number of possible links between them [45] (Fig. 3a).

The clustering coefficient of a network, $C(G)$, is the average over the clustering coefficient of all nodes (see Eq. (4) in Table 1). Clustering coefficient values lies in the range from 0 to 1. Densely interconnected neighbors yield high clustering coefficients, while sparsely interconnected neighbors return low coefficients. Networks with high clustering coefficient have larger resilience.

Another way to measure the clustering is by transitivity $T(G)$ (sometimes also called clustering coefficient) (see Eq. (5) in Table 1). The original definition of transitivity comes from social science and indicates how much a network is clustered [44]. This formulation denotes the fraction of triples of nodes present in a network that form a cluster. Transitivity and the clustering coefficient capture the same intuition of clustering, but they are of different quantities [44].

3.3. Characteristic path length

Another fundamental property of brain networks is functional integration, which indicates how integrated a network is and, thus, how easily information flows [46]. A common approach to estimate the potential for functional integration between nodes is based on the concept of path length [46] (Fig. 3b).

The shortest path length $d(i,j)$ (also called distance or geodesic path) is defined as the minimum number of links that must be traversed to go from node i to node j [45] (see Eq. (7) in Table 1). By definition, $d(i,j) \geq 1$. Therefore, $d(i,j) = 1$ if i and j are neighbors, whereas $d(i,j) \to \infty$ if the nodes are disconnected. The average of the shortest path length between all pairs of nodes in a network is called the characteristic path length (L). Small characteristic path length implies stronger potential for integration [46].

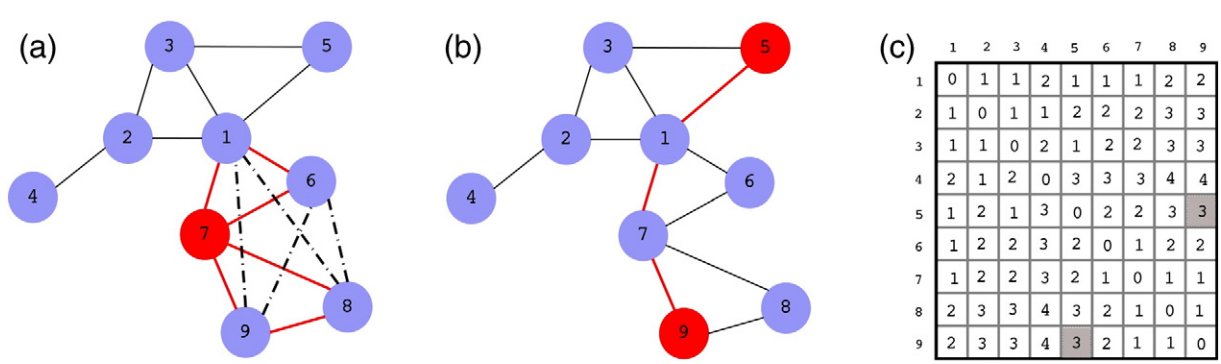
3.4. Cost

It is reasonable to admit that the greater the number of links, the higher its integration and, consequently, its efficiency. In contrast, in any real network, there is a price to pay for each additional link. This price is measured by the density of links, which is known as cost or connection probability (see Eq. (3) in Table 1). If the cost is low, the network is considered economic [44].

3.5. Efficiency

Assuming a parallel flux of information, the efficiency of communication between two nodes is defined to be inversely proportional to the shortest path length, $\varepsilon(i,j) = 1/d(i,j)$ [47]. Consistently, $d(i,j) \to \infty$, return $\varepsilon(i,j) = 0$, and the efficiency is maximum, $\varepsilon(i,j) = 1$, when $d(i,j) = 1$. The efficiency of a set of nodes is the sum of the efficiencies of all node pairs, normalized by maximal number of links $N(N-1)/2$.

When the set of nodes includes all nodes, then the resulting efficiency is known as the global efficiency, E_{global} (see Eq. (8) in Table 1) [7]. Local properties can also be characterized by taking subgroups only with neighbors of each node in choosing the set of nodes. The average of all such subgroups of the network is the local efficiency, E_{local}, of the network (see Eq. (6) in Table 1). The local efficiency reveals how



	1	2	3	4	5	6	7	8	9
1	0	1	1	2	1	1	1	2	2
2	1	0	1	1	2	2	2	3	3
3	1	1	0	2	1	2	2	3	3
4	2	1	2	0	3	3	3	4	4
5	1	2	1	3	0	2	2	3	3
6	1	2	2	3	2	0	1	2	2
7	1	2	2	3	2	1	0	1	1
8	2	3	3	4	3	2	1	0	1
9	2	3	3	4	3	2	1	1	0

Fig. 3. Cluster coefficient and path Length. (a) A representation of the cluster coefficient of node 7 (red) and all possible connections between its neighbors by a dashed line is displayed. (b) A graphical representation of shortest path length between nodes 5 and 9 (thick line) and (c) its path length matrix (or distance matrix).

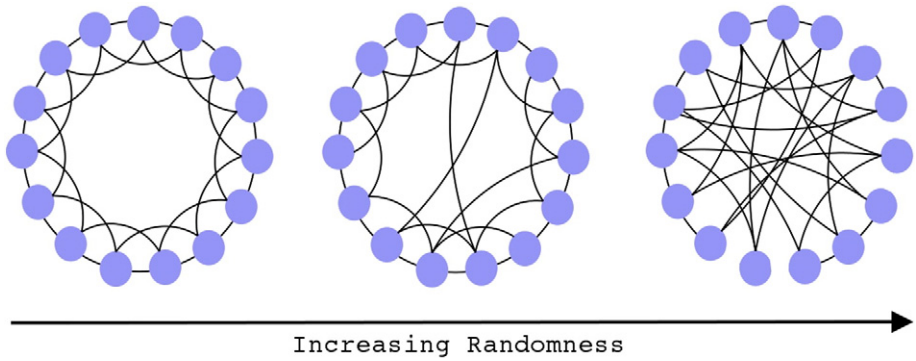


Fig. 4. The Watts–Strogatz model of the small-world. The network on the left represents a ring lattice with circular boundary conditions. The initial configuration connections are randomly rewired with a given rewiring probability p. For $p = 0$ (no rewiring), the network retains its regular lattice topology. For $p = 1$, the network is completely random (network at right), and all lattice features disappear. Intermediate values of p result in networks that consist of a mixture of random and regular network (at the center where the small-world phenomenon emerges) [45].

efficient the communication is between the first neighbors of a node when it is removed [47].

Global efficiency is a measurement of functional integration, while local efficiency is associated with functional segregation. They are alternative metrics to clustering coefficient and characteristic path length [48].

3.6. Random networks

Random networks are commonly defined as networks in which the links are randomly distributed [1]. The most famous model of random networks was proposed by Erdös and Rényi and consists of networks such that the links are distributed randomly with constant probabilities of connection between two nodes (see Ref. [2] for details). Thus, by varying the numbers of nodes and probabilities of connection, different types of networks can be constructed [42]. However, it is only one among many possible random models. In a more general definition, the random network is a model in which some chosen parameters assume fixed values, while other parameters of network are free to vary randomly [49].

3.7. Small-world

A ubiquitous network architecture that deserves attention is the small-world network. The concept of small-world emerged in the late 1960s, in a social system study by Stanley Milgram, which led to the popular expression "six degrees of separation" [50]. It characterizes how efficiently the information is exchanged over a network [47].

The idea was prominently inserted in the context of complex networks by a study of Watts and Strogatz, in which it was shown that real networks are neither completely regular nor completely random, but stays between these two extremes [45] (Fig. 4). Regular networks can be seen as the opposite of random graphs. They present an ordered structure with high clustering and high characteristic path length, while random graphs are characterized by a low clustering coefficient and low path length (Fig. 4). Therefore, in order for a network to be classified as small-world, it needs a characteristic path length similar to a random network $L \sim L_{rand}$ but a substantially higher clustering coefficient, $C \gg C_{rand}$ [6].

In an attempt to refine this measurement, Humphries and Gurney proposed the "small-worldness" metric, σ (see Eq. (9) in Table 1). In this formulation, the network is classified as small-world if $\sigma >> 1$ [51]. It is important to emphasize, however, that in order to compare small-world behavior of several networks, it is indispensable to analyze clustering coefficient and characteristic path length apart, since σ cannot capture opposite behaviors such as a concurrent increase of $L(G)$ with a decrease of $C(G)$. This architecture is of particular interest for neuroscience as brain networks of different scales exhibit small-world behavior [52].

The general idea behind the use of complex network to fMRI data is to compute the abovementioned metrics in resting state fMRI experiments. However, the implementation of such strategies is not trivial. Therefore, the next sections guide the reader throughout an example on how this is commonly conducted.

4. Complex networks and fc-fMRI

A conventional resting state fMRI protocol, as described above, is the most frequently used approach to investigate complex networks in the scenario of functional neuroimaging.

Once acquired, images have to be preprocessed using standard fMRI steps, which include slice-timing correction, head motion correction, and spatial smoothing. Moreover, most of the time, they are normalized to an anatomical standard space, such as the Montreal Neurologic Institut (MNI 152) [53].

The nodes are typically defined according to three approaches: image voxels [54], segregation based on brain functional division, and, most commonly, anatomical brain divisions [46]. In the anatomical approach, brain atlases (Fig. 5a) can be used to segment the brain into a number of subregions, which will constitute the nodes. A possible and frequently used atlas is the *Harvard-Oxford cortical and subcortical structural atlas* (with a threshold usually set at 25%). It is part of the FSL package (FMRIB Software Library, www.fmrib.ox.ac.uk/fsl), and it parcellates the brain into 110 anatomical regions (nodes) (Fig 6b).

On the other hand, the links are usually assembled as measures of functional or effective connectivity [5] between pairs of nodes. These quantities come from the time courses computed from the average HRF at each node (Fig. 6c). It is important to note that these time series are usually contaminated by a number of confounders, which come mainly from head motion, the signal from the white matter, the cerebrospinal fluid (CSF), and the global average signal[1] [55] that are thought to account for physiological noise (cardiac and respiratory). Hence, time series representing these quantities have to be regressed out from the data [56]. The remaining signal should be filtered either by a band-pass filter or by a wavelet transform. Often, the wavelet approach is chosen as biological data usually demonstrate scale invariant or fractal properties [57]. For this purpose, one can use the maximum overlap discrete wavelet transform (MODWT —www.atmos.

[1] The use of the whole-brain average signal, also called global signal, is a controversial step, and its validity is under discussion as it may introduce artifactual negative correlations.

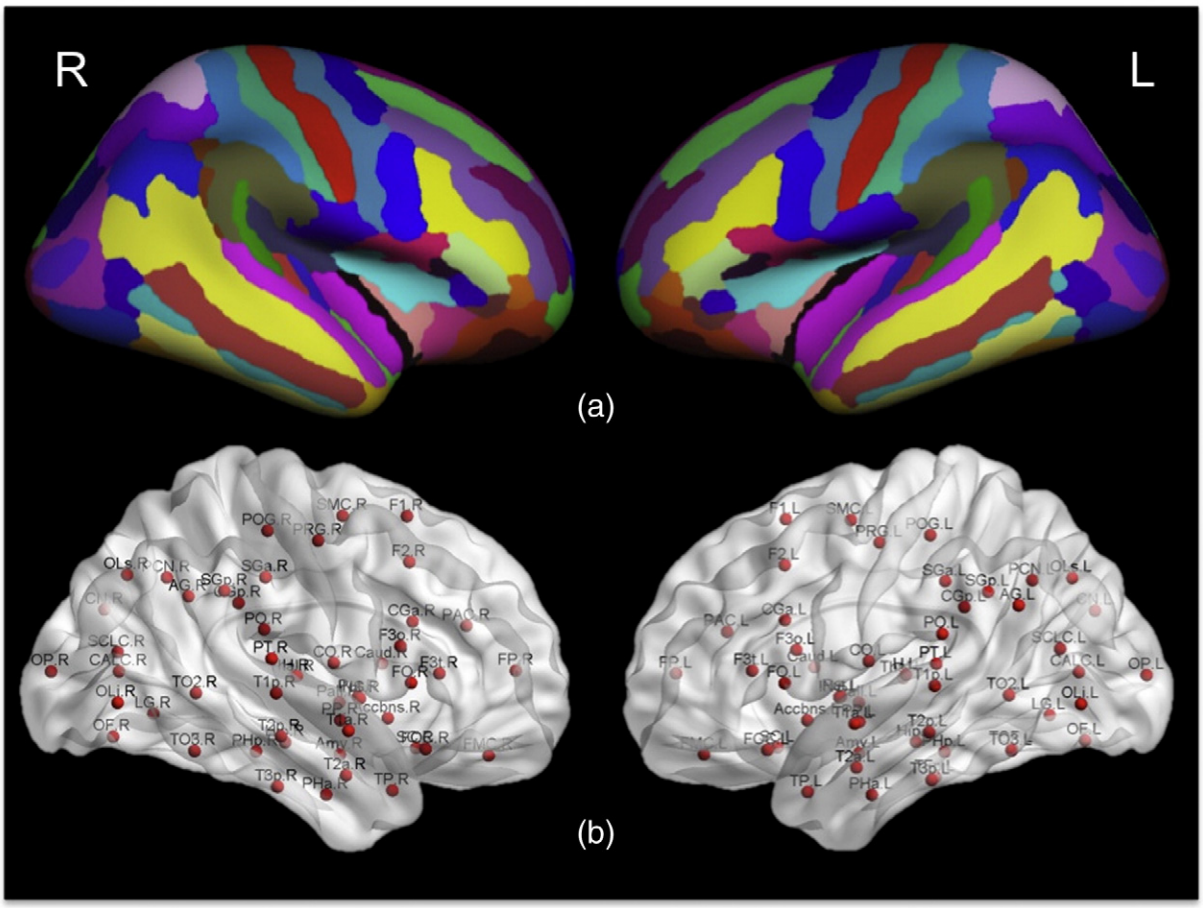


Fig. 5. Example of nodes based on anatomical regions. (a) The Destrieux atlas [76] was overlaid onto an inflated brain surface using the software FreeSurfer version 5.3.0 (https://surfer.nmr.mgh.harvard.edu/). (b) This atlas was used to define the nodes of the brain network.

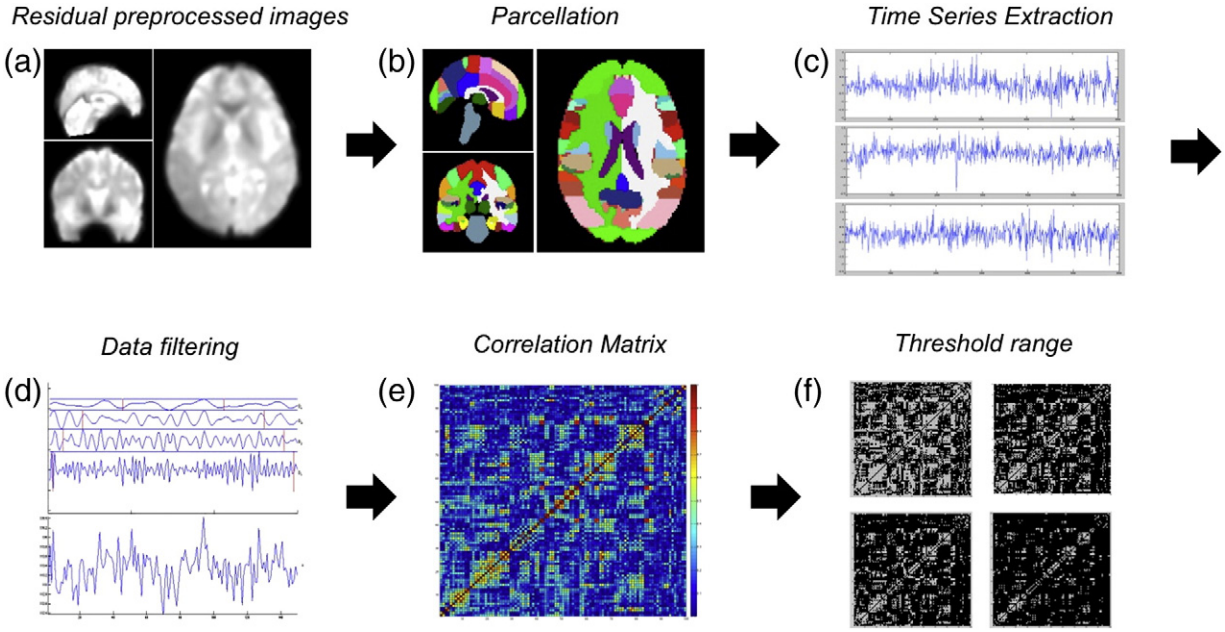


Fig. 6. A step by step illustration on how graphs from fMRI data can be generated. (a) *Residual preprocessed images* —preprocessed methods as slice-timing correction, head motion correction, spatial smoothing, and normalization are first carried out on raw fMRI data. (b) *Parcellation* —a brain atlas can be used to divide the brain in subregions. (c) *Time series extraction* —mean time series are extracted from each region to be further used to calculate the links of the network. (d) *Data filtering* —to select the frequency band of interest, the remaining signal can be filtered either by a band-pass filter or by a wavelet transform. (e) *Correlation matrix* —the correlation matrix can be constructed based on calculation of Pearson correlation coefficients between pairs of regions. (f) *Threshold range* —for each connection probability value, a binary matrix can be constructed. Smaller threshold values increase the connection probability, and more links are added to the network (more white dots), while, bigger threshold values result in less connections and, consequently, decreased connection probability (more black dots).

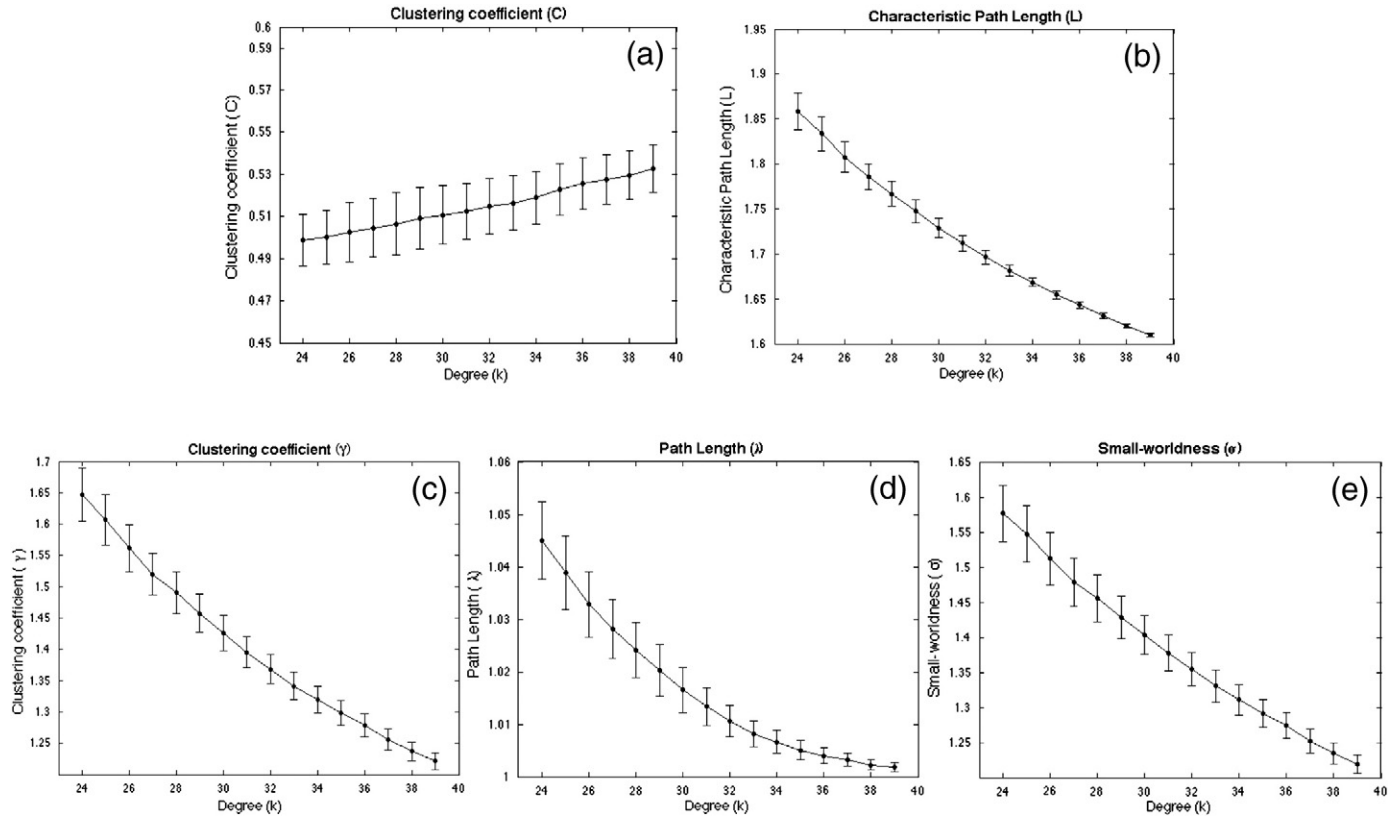


Fig. 7. Examples of topological measures. (a) Clustering coefficient (C), (b) characteristic path length (L), (c) normalized clustering coefficient ($\gamma = C/C_{rand}$), (d) normalized path length ($\lambda = L/L_{rand}$), and (e) small-worldness ($\sigma = \gamma/\lambda$). Metrics were calculated for a healthy group (7 subjects) as a function of the degree *k*. Error bars indicate standard error of the mean.

washington.edu/~wmtsa/) to divide the time series into different frequency bands (Fig. 6d). Typically, the signals are filtered in the range of approximately 0.01 to 0.1 Hz [58]. After these preprocessing steps, the remaining signal will be used to construct the links, based, for instance, on functional connectivity (Pearson correlation, see Eq. (10) in Table 1) measured between pairs of signals (nodes).

The result is an $N \times N$ (N = total number of nodes) symmetric correlation matrix (Fig. 6e), which represents the correlation coefficient (r_{xy}) between the time series of pairs of nodes, *i* and *j*. This weighted matrix can be directly used to construct a network. However, commonly this matrix is thresholded in order to obtain a binary matrix.[2] The threshold choice is of fundamental importance as it defines the topology of the network. To date, there are two common approaches for this choice [59]: (*i*) to choose a single and optimal threshold to apply to all subjects and (*ii*) to choose a range of values and describe the network proprieties as a function of the mean degree of the network.

Considering this last approach, usually the threshold range is chosen as to guarantee a small-world network regime [7]. This choice may be based on the correlation coefficient (r_{xy}) or on the connection probability (cost). In either case, the lowest threshold should be chosen to assure that the network does not have disconnected nodes. On the other end, the upper threshold should not include networks that behave like a random network. To meet this second requirement, the network needs a lower global efficiency and a larger local efficiency than its respective random network [7,60]. Therefore, individually constructed random networks are compared with the subject's adjacent matrix at a specific threshold, defining the range of interest. The result is a series of binary matrices, one for each threshold (Fig. 6f).

With all matrices in the selected range for all subjects, it is possible to calculate the desired network metrics by a number of software packages, such as the brain connectivity toolbox (www.brain-connectivity-toolbox.net [47]). Every subject will have a specific value for the metric of interest. Thus, group averages may be computed at each threshold (Fig. 7), and, further, a statistical comparison between different groups can be computed. Besides cluster coefficient (C) and path length (L), comparisons are also based on normalized values of clustering coefficient ($\gamma = C/C_{rand}$) and path length ($\lambda = L/L_{rand}$), which allows the computation of "small-worldness" (σ).

Finally, individual network topologies can be visualized for a specific threshold, which generally represent an overview of the calculated metrics. There are a number of programs designed for this purpose, such as the BrainNet Viewer (www.nitrc.org/projects/bnv) (Fig. 8).

Approaches like the ones just described have been used by several studies and have found complex network changes related to different conditions in basic neuroscience [4], neurology [8], and psychiatry [7]. For instance, graph theoretical analysis has been applied to patients with partial seizures and generalized absence seizures in order to measure topologically and organizationally changes of brain networks. To date, most of these analyses were conducted using intracranial and scalp EEG data [61–70]. Recently, a few studies have started to use these strategies on functional neuroimaging data, such as fMRI [6,71,72].

5. Complex network, fMRI, and epilepsy

Zhang et al. used diffusion tensor imaging and resting state fMRI to analyze and compare anatomical and functional whole-brain network of healthy subjects and patients with generalized tonic–clonic seizures (GTCS) [6]. In general, their findings suggest a less-optimized network organization in the patient group during interictal activity.

[2] If $r_{xy} > Th$, $r_{xy} = 1$; otherwise $r_{xy} = 0$. r_{xy} with $p < 0.05$ are also turned to zero.

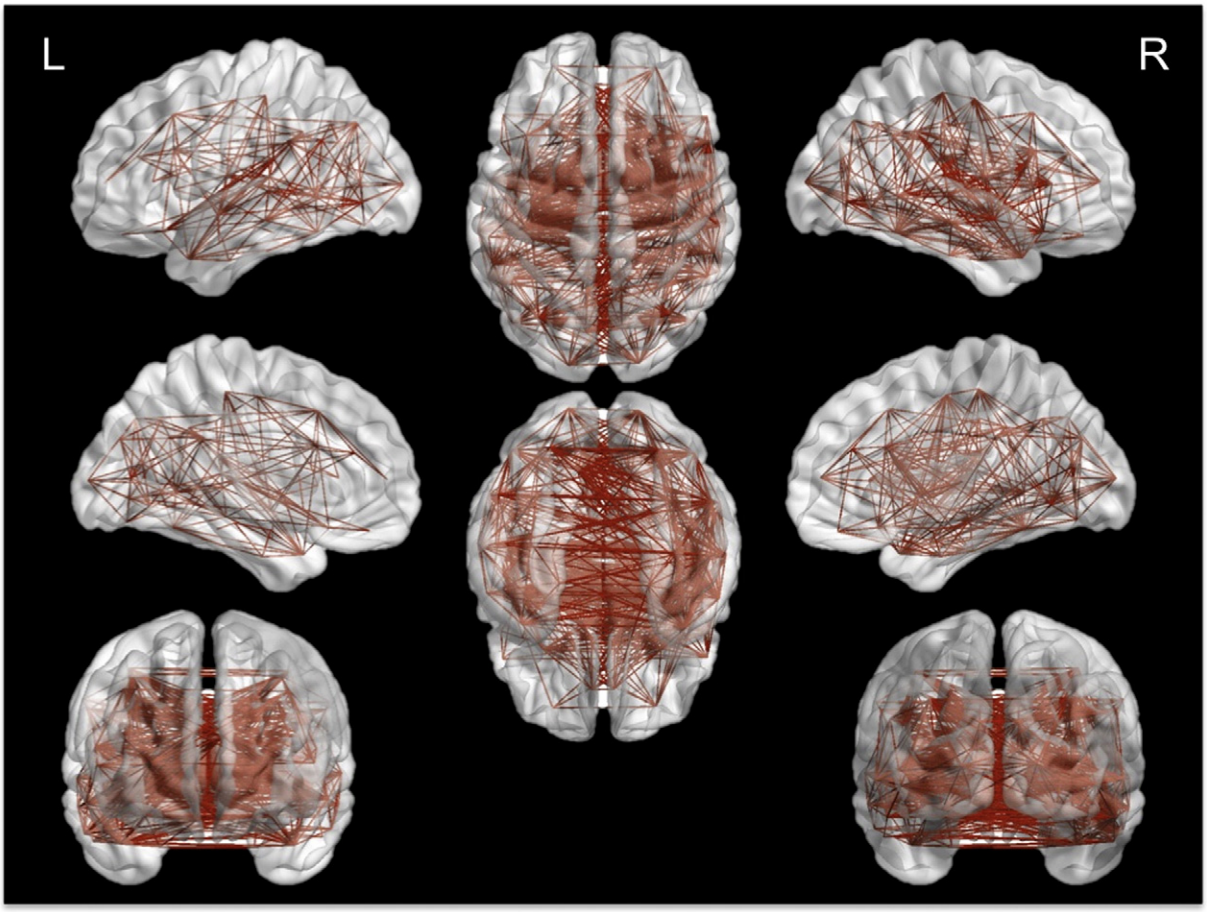


Fig. 8. Illustrative brain network of a patient with focal seizures in the left hemisphere.

This is represented by a decrease in "small-worldness" due to a decrease in the normalized clustering coefficient, with no change in normalized characteristic path length. On the other hand, patients with GTCS showed increased total connection strength in functional but not in structural connectivity. Furthermore, at the nodal level, they found a decrease in functional nodal topological properties in the brain default mode network (DMN). The amygdala showed an increase not only in functional nodal degree but also in efficiency and centrality. More interestingly, these changes were positively correlated with the duration of epilepsy. It is also worth noting that coupling of functional and structural connectivity networks was significantly decreased in IGE–GTCS, and it was negatively correlated with duration of epilepsy.

Liao et al. [71] also used resting state fMRI data to investigate functional connectivity in mesial temporal lobe epilepsy during interictal periods and, in line with Zhang and colleagues [6], observed that DMN regions have a significant decreased number of connections to other regions. Patients showed no change in normalized clustering coefficient and a decrease in normalized characteristic path length. This study also found a negative correlation of the functional connectivity between the right inferior frontal gyrus (opercular) and the left inferior frontal gyrus (triangular) with the epilepsy duration, suggesting a relationship between the decreased connectivity and the functional impairment associated with epilepsy duration.

Vlooswijk et al. [72] used fMRI with a word generation paradigm and an intelligence test to investigate brain network properties and their association with intellectual decline in patients with frontal and temporal lobe epilepsy. They observed lower values of the normalized cluster coefficient and global and local efficiency in patients when compared with healthy controls. The authors showed a diffuse disruption of small-worldness in the patient group. Interestingly, they observed that topology changes in patients with epilepsy were associated with a decline in intellectual abilities, although it is difficult to distinguish the effect of antiepileptic drugs from epilepsy itself.

6. Final remarks

Although complex networks have been successfully used in the fMRI context, there are several limitations that should be taken into account [27]. At each stage of the network construction, choices are made that determine the final metric values.

For instance, the number of regions, their sizes, and specific locations have to be chosen when nodes are constructed based on anatomy, and this will bias statistical tests [59,73]. Link representation should also be observed. They are often defined by functional connectivity measurements using a Pearson correlation coefficient. This approach, although easily implemented and commonly used in several studies, does not take into account the contribution of all other regions when calculating the pairwise correlation. An alternative approach has been the use of a partial correlation [27,74]. Another critical point is the choice of threshold. It is clear that it determines the regime of network. However, it also influences the statistical analysis [75].

Finally, and most importantly, metrics can be extracted from different conditions and diseases, using various tactics, leading to observed differences between groups. However, what are their interpretation and meaning? In fact, we believe that this question lies at the core of

the future perspective of complex network use for fMRI data and to brain sciences as a whole.

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APÊNDICE D – Artigo publicado durante o doutorado

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RESEARCH ARTICLE

The Psychedelic State Induced by Ayahuasca Modulates the Activity and Connectivity of the Default Mode Network

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Citation: Palhano-Fontes F, Andrade KC, Tofoli LF, Santos AC, Crippa JAS, Hallak JEC, et al. (2015) The Psychedelic State Induced by Ayahuasca Modulates the Activity and Connectivity of the Default Mode Network. PLoS ONE 10(2): e0118143. doi:10.1371/journal.pone.0118143

Academic Editor: Dewen Hu, College of Mechatronics and Automation, National University of Defense Technology, CHINA

Received: May 28, 2014

Accepted: December 11, 2014

Published: February 18, 2015

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Data Availability Statement: Data have been deposited to the Harvard Dataverse Network, DOI: 10.7910/DVN/28619 (http://thedata.harvard.edu/dvn/dv/neuroimago).

Funding: Contract grant sponsor: The Brazilian Federal Agencies: CNPq, CAPES; FINEP; The Sao Paulo State financial agency (FAPESP). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Abstract

The experiences induced by psychedelics share a wide variety of subjective features, related to the complex changes in perception and cognition induced by this class of drugs. A remarkable increase in introspection is at the core of these altered states of consciousness. Self-oriented mental activity has been consistently linked to the Default Mode Network (DMN), a set of brain regions more active during rest than during the execution of a goal-directed task. Here we used fMRI technique to inspect the DMN during the psychedelic state induced by Ayahuasca in ten experienced subjects. Ayahuasca is a potion traditionally used by Amazonian Amerindians composed by a mixture of compounds that increase monoaminergic transmission. In particular, we examined whether Ayahuasca changes the activity and connectivity of the DMN and the connection between the DMN and the task-positive network (TPN). Ayahuasca caused a significant decrease in activity through most parts of the DMN, including its most consistent hubs: the Posterior Cingulate Cortex (PCC)/Precuneus and the medial Prefrontal Cortex (mPFC). Functional connectivity within the PCC/Precuneus decreased after Ayahuasca intake. No significant change was observed in the DMN-TPN orthogonality. Altogether, our results support the notion that the altered state of consciousness induced by Ayahuasca, like those induced by psilocybin (another serotonergic psychedelic), meditation and sleep, is linked to the modulation of the activity and the connectivity of the DMN.

Introduction

The overwhelming experience induced by psychedelics such as LSD, psilocybin, mescaline and N,N-dimethyltryptamine (DMT) share common characteristics [1]. From the neuropsychological perspective, they are all substances known to induce complex mystical experiences, modulating the sensory, perceptual, cognitive and autonomic systems, as well as emotional processes. A renowned effect of these substances is the increased vividness of both perception and internally generated mental images, therefore the name hallucinogens [1,2].

Competing Interests: The authors have declared that no competing interests exist.

In order to gain insight into the neurophysiology underlying mental imagery induced by psychedelics, we have used functional Magnetic Resonance Imaging (fMRI) to investigate the effects of Ayahuasca, a powerful psychedelic traditionally used by Amazonian Amerindians, and then spread to Western countries, occupying the core of some Brazilian syncretic religions [3,4]. This beverage is produced from the decoction of two different plants: the *Psychotria viridis* and the *Banisteriopsis caapi*. The first one contains the psychedelic tryptamine N,N-dimethyltryptamine (DMT) that binds to serotonin and sigma-1 receptors [4,5]. The second is rich in beta-carboline alkaloids, particularly harmine, tetrahydroharmine (THH), and harmaline. Harmine and harmaline are potent monoamine oxidase inhibitors (MAOi) and THH acts as a mild selective serotonin reuptake inhibitor and a weak MAOi [6]. During execution of a mental imagery task, we found that Ayahuasca selectively modulates frontal, temporal and occipital brain networks associated with intention, memory and vision [7]. The effects include significant increased activity of the primary visual cortex, in tight correlation with psychometric changes [7].

In addition to the amplification of mental imagery, the experience induced by Ayahuasca involves sedation, gastrointestinal distress, changes of spatiotemporal scaling, dissociation, sense of well-being, insights, feelings of apprehension, and, very notably, increased introspection (the inspection of one owns thoughts and feelings) [2,8–11]. A group of brain regions that has lately received considerable attention due to its consistent association with internally oriented mental processes is the Default Mode Network (DMN) [12–17]. This network presents increased activity while one is at rest, in comparison with activity levels detected during execution of externally oriented cognitive task [14,18]. Accordingly, mind wandering, a private, continuous and often unnoticed phenomenon, is known to involve the DMN [13,19,20]. Indeed, functional neuroimaging studies have consistently associated the activity of the DMN with key components of mind-wandering, such as remembering the past and planning the future [21,22].

The activity of the DMN is sensitive to a number of tasks, states of consciousness and psychiatric conditions. On one end of the spectrum, DMN activity is increased in schizophrenia [23], depression [12], Parkinson's disease [24], social phobia [25], and by tetrahydrocannabinol administration (THC) [26]. On the other end, DMN activity is reduced in autism [27], Alzheimer's disease [28], during hypnosis [29], meditative states [17], and also by psilocybin intake [16].

Resting state fMRI (rs-fMRI) has allowed the evaluation of how DMN areas interact with each other, for instance by means of functional connectivity (fc-fMRI) measurements [30]. Indeed, this technique has proven to be sensitive enough to detect connectivity changes between DMN nodes associated with a number of altered states of consciousness, such as sedation, sleep, meditation, and psilocybin [31–35]. Furthermore, rs-fMRI has lead to the suggestion that the DMN exhibits an intrinsic competitive behavior with another brain network related to externally oriented tasks, known as task-positive network (TPN) [36]. The anti-correlation between DMN and TPN found in rs-fMRI indicates that when the signal of one network is increased, the signal of the other tends to decrease [36,37].

Although Ayahuasca intake increases introspection [11], it is expected that it also reduces DMN activity, analogous to psilocybin and meditation. Hence, the first aim of this study was to investigate how Ayahuasca interferes with the DMN activity, using an active task. We also used rs-fMRI to investigate how Ayahuasca experience interferes with functional connectivity between different DMN regions. Lastly, we analyzed the impact of Ayahuasca over the TPN-DMN orthogonality, to assess whether part of the Ayahuasca experience comes from an increased overlap between internally and externally oriented attention.

Materials and Methods

Ethics Statement

The work was approved by the Ethics and Research Committee of the University of Sao Paulo at Ribeirao Preto (N° 14672/2006), and written informed consent was obtained from all subjects that participated in this study.

Subjects

Ten healthy volunteers (5 males; mean age of 29 years, ranging from 24 to 48 years) with at least 5 years of regular (twice a month) Ayahuasca use were recruited from Santo Daime church to participate in this study. They were all right-handed, had no comorbid history of neurological or psychiatric disorder as assessed by DSM-IV structured interview, and were not under medication for at least 3 months prior to the study. Subjects were abstinent from caffeine, nicotine and alcohol prior to scanning session. Data from one volunteer was excluded from analyses due to excessive head movement. The final dataset included 9 subjects (five women). All presented limited motion artifact: before (0.14 ± 0.05 mm), and after Ayahuasca intake (0.09 ± 0.11 mm), which were not significantly different (Wilcoxon paired test—p-value = 0.13). An additional group of 26 control individuals (11 males; mean age: 37.39 years) also participated in this study in order to define the Regions of Interest (ROI) of the DMN.

Experimental design

Subjects underwent two fMRI sessions, before and 40 min after Ayahuasca intake (2.2 mL/kg of body weight). The Ayahuasca used was provided by the Santo Daime church and contained 0.8 mg/ml of DMT and 0.21 mg/ml of harmine. Presence of harmaline was not detect under the chromatography threshold of 0.02 mg/mL [7]. In each session subjects performed two protocols. The first one was used to evaluate DMN activity. It consisted of a verbal fluency task, designed as a block paradigm, which alternated six blocks of rest with five blocks of silently generated words starting with a cued letter (M, A, E, C and S, one letter for each block). Each block lasted 27.5 seconds. During rest periods the subjects were asked to think of a white wall. The second protocol was a conventional rs-fMRI acquisition, designed to evaluate the functional connectivity of the DMN. During acquisition, subjects were asked to keep their eyes closed and to hold their head as still as possible. To evaluate subjective effects caused by Ayahuasca, two psychiatric scales were applied at time 0 (baseline), 40 minutes, 80 minutes and 200 minutes after Ayahuasca ingestion: the Brief Psychiatric Rating Scale (BPRS), and the Young Mania Rating Scale (YMRS) [7].

Image acquisition

Images were acquired in a 1.5 T scanner (Siemens, Magneton Vision). For the verbal fluency task, a total of 66 volumes were acquired, each with 16 slices, using an EPI sequence with the following parameters: TR = 4600 ms; TE = 66 ms; FOV = 220 mm; matrix 128 x 128; voxel dimensions of 1.72 mm x 1.72 mm x 5.00 mm. For the resting state protocol, 150 volumes were acquired with the same parameters used in the block paradigm except for the TR, which was set to 1700 ms, and a matrix of 64 x 64. Whole brain high resolution T1-weighted images were also acquired, consisting of 156 contiguous sagittal slices using a multiplanar reconstructed gradient-echo sequence, with the following parameters: TR = 9.7ms; TE = 44ms; flip angle 12°; matrix 256 x 256; FOV = 256 mm, voxel size = 1 mm x 1 mm x 1 mm.

fMRI pre-processing

Preprocessing of the functional dataset was performed in FSL software (Analysis Group FRMIB, Oxford, UK), and included slice-timing correction, head motion correction, and spatial smoothing (Gaussian kernel, FWHM = 5 mm). EPI were first registered to the anatomical images using a rigid body transformation (6 DOF), and then normalized to the Montreal Neurologic Institute (MNI) 152 template using a linear transformation (12 DOF).

DMN ROI definition

To avoid double dipping, i.e., the use of the same data set for selection and selective analysis [38], the DMN Regions of Interest (ROI) were defined based on data from the control group, who performed the same verbal fluency task. Statistical analysis was conducted in SPM5 (Statistical Parametric Mapping, Wellcome Trust Centre for Neuroimaging, London, UK). For both control and experimental groups, fMRI time courses were high-pass filtered at 0.015 Hz and analyzed using the General Linear Model (GLM) with a regressor modeling the task periods, convolved with a canonical hemodynamic response function (HRF). For each subject, a contrast was applied (rest > task) using t-test. The contrasts then entered into a second level random effect analysis to create statistical maps from the groups of subjects ($q[FDR] < 0.01$, cluster size of 50 contiguous voxels).

The DMN mask used was based on the intersection of the statistically significant voxels with the following nine brain areas consistently associated with the DMN [39]: the anterior cingulate cortex (ACC), the posterior cingulate cortex (PCC), the precuneus (PC), the medial prefrontal cortex (mPFC), the left middle frontal gyrus (LMFG), the left and right middle temporal gyrus (LMTG and RMTG), and the left and right inferior parietal lobule (LIPL and RIPL).

Effects of Ayahuasca over the DMN

Experimental group maps were compared between conditions. For each subject, a contrast was applied (rest > task) using a t-test. The contrasts then entered into a second level fixed effect analysis in order to perform a paired comparison of the two instants: before (DMN-Before) and after (DMN-After). Statistical maps presented onto inflated cortical surfaces were constructed using the FreeSurfer package (version 5.0.0, http://surfer.nmr.harvard.edu). Furthermore, averaged β-values and standard deviations were extracted from the nine ROI in the experimental group, both before and after Ayahuasca intake. Post-hoc statistical analyses were performed in GraphPad Prism 4.00 (GraphPad Software, San Diego, CA, EUA), using the Wilcoxon paired test, corrected for multiple comparisons by the total number of ROI (nine).

Mean individual β-values were also extracted for each ROI, before and after Ayahuasca ingestion. They were used to compute the correlation between individual β-values change ($\Delta\beta = \beta_{after} - \beta_{before}$) and the individual scores of the psychometric scales at 80 min (right after MRI scanning), corrected for multiple comparisons by 12 (6 ROI x 2 scales).

fMRI functional connectivity (fc-fMRI)

Images from the resting state paradigm were used to evaluate seed-based functional connectivity (fc-fMRI), in the software REST (Resting-state fMRI Data Analysis Toolkit, version 1.7 [40]). To reduce spurious correlations, averaged time courses from white matter, cerebrospinal fluid, global signal, and the six rigid-body realignment parameters were regressed out using SPM. The residual time series were then filtered between 0.01 and 0.08 Hz. One seed (10 mm radius sphere) was defined at the two main hubs of the DMN: PCC (MNI coordinates: -4, -47, 45) and mPFC (MNI coordinates: 0, 51, -14), according to [36]. Average time series extracted from these two

seeds (both before and after Ayahuasca) served to calculate their temporal correlations with every other voxel in the brain. Results were expressed in terms of voxel-wise Pearson coefficients, z-Fisher transformed. A group analysis was performed concatenating the z-maps of both seeds and comparing the sessions (before and after Ayahuasca) through a paired t-test. Additionally, statistical differences were assessed considering the z-maps of each seed separately. Statistical differences were evaluated only in the nine ROI previously defined.

DMN x TPN orthogonality

Herein we used a classical approach to define the TPN [36], where three seed regions (10 mm radius spheres) are selected, each one centered at: intraparietal sulcus—IPS (MNI coordinates: -25, -54, 53), frontal eye field—FEF (MNI coordinates: 29,-7, 52) and middle temporal region—MT (MNI coordinates: -47, -72, 1). The same filtered residual time series from the fc-fMRI analysis were used to construct the correlation maps. Before Ayahuasca intake, correlations maps were built, one for each seed, and Fisher's z-transformed. The z-maps of the three seeds entered into a one-sample t-test, and then binarized to obtain a TPN mask using a threshold of $p < 0.001$. DMN was defined based on PCC and mPFC seeds as described in the fc-fMRI section. This mask and the DMN mask were used to extract average time series for each subject, from which Pearson's correlation coefficients were calculated between DMN and TPN for both conditions (before and after) separately. Statistical differences were assessed using a paired t-test, and threshold was set at $p < 0.05$. Moreover, as it has been suggested that global signal regression may create false negative correlations [41,42], data was also analyzed keeping the global signal in the pre-processing step.

Results

The mask that defined the DMN, outlined from a control group, is shown in S1 Fig. Based on these ROI, the signal of the DMN changed significantly when comparing rest with task periods, both before and after Ayahuasca intake (S2 Fig.). Statistical comparison between maps obtained for the two moments (before vs. after) revealed a significant signal decrease for most parts of the DMN as a result of Ayahuasca intake (Fig. 1). Two ROIs (LMFG and LMTG) presented a significant signal increase after Ayahuasca intake. These structures are known to be involved in language processing (Binder, et al., 1997), and since we used a verbal fluency task it is possible that they are also engaged during task execution, therefore confounding the interpretation of these results.

A further ROI inspection confirmed that Ayahuasca effects are accompanied by statistically significant reduction of activity for most parts of the DMN ($p < 0.01$, Bonferroni corrected for

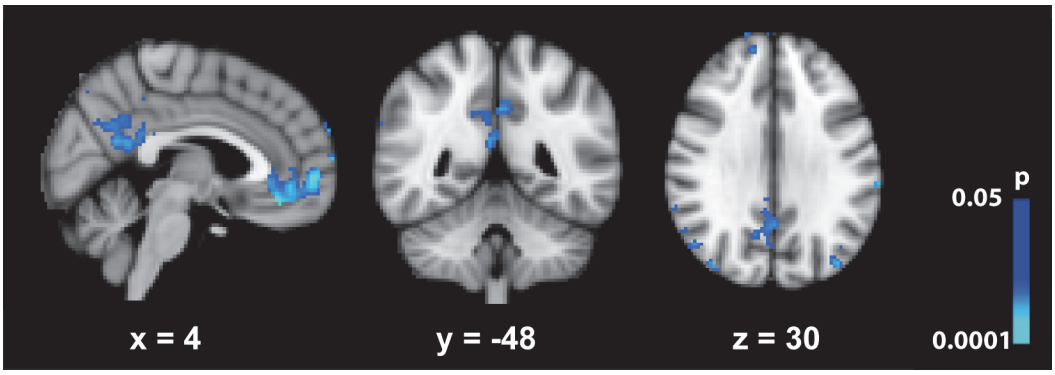

Fig 1. Statistical maps showing regions where BOLD signal of the DMN (rest > task) decreases after Ayahuasca ingestion. $P < 0.05$ uncorrected.

doi:10.1371/journal.pone.0118143.g001

Table 1. Changes in DMN (rest > task) BOLD signal following Ayahuasca intake.

		β-values Before Ayahuasca		β-values After Ayahuasca			
ROI	Nvoxel	Mean	SD	Mean	SD	P value	
ACC	606	0.4193	0.2797	0.3188	0.1497	< 0.0001	*
PCC	2058	0.6409	0.3636	0.5378	0.2397	< 0.0001	*
MPFC	2963	0.551	0.3701	0.4211	0.2854	< 0.0001	*
RIPL	3216	0.5226	0.2997	0.4620	0.2612	< 0.0001	*
LIPL	1749	0.4464	0.2650	0.3795	0.2392	< 0.0001	*
LMFG	98	0.0798	0.0830	0.2140	0.1419	< 0.0001	*
RMTG	1731	0.1858	0.1631	0.1860	0.1309	0.7996	
LMTG	1028	-0.0211	0.1868	0.0651	0.1740	< 0.0001	*
PC	3929	0.8131	0.3491	0.7818	0.3482	< 0.0001	*

Nvoxel = number of voxels in each ROI, mean and standard deviation of β-values before and after Ayahuasca ingestion (in % BOLD signal change).
* Indicates significant differences after Ayahuasca ($p<0.0011$, corresponding to $p<0.01$ corrected for multiple comparisons by the number of ROI).

doi:10.1371/journal.pone.0118143.t001

multiple comparisons). S3 Fig. shows the average β-values and standard error for both sessions (before and after Ayahuasca ingestion), in each of the 9 ROI. Significant decreased β-values were observed in ACC, PCC, MPFC, PC and bilateral IPL (S3 Fig., Table 1).

The correlation analysis between individual Δβ and corresponding psychiatric scales revealed a tendency for significant negative correlations between YMRS scores 80 minutes after Ayahuasca ingestion only at ACC ($r = -0.78$, $p = 0.072$, Bonferroni corrected for 12).

To inspect how the different regions of the DMN relate to each other, fc-fMRI maps were constructed before and after Ayahuasca ingestion (S4 Fig.). The direct statistical comparison of these maps exhibited a significant functional connectivity decrease within the PCC/Precuneus (Fig. 2A). By analyzing each seed independently, it appears that most of the contribution for the observed connectivity decrease is driven by the PCC (Fig. 2B). We did not observe significant connectivity changes induced by Ayahuasca when explicitly evaluating the correlation between mPFC and PCC seeds ($r^2 = 0.13$; -0.02, before and after Ayahuasca ingestion respectively, $p = 0.12$).

In addition to investigating the changes in the activity and connectivity of the DMN, we also analyzed its interaction with the TPN. In the first analysis, following most studies that compute DMN-TPN correlations, the global signal was regressed out during data pre-processing. Results are presented in Fig. 3. Fig. 3A shows the masks used for TPN (red) and DMN (blue). Fig. 3B shows the expected anti-correlation between TPN and DMN. There was no statistically significant difference in DMN-TPN orthogonality when comparing before and after Ayahuasca ingestion (Fig. 3B).

As increasing evidences have been pointing out to the fact that regression against global signal induces spurious negative correlation [41–43], we re-analyzed this dataset without global signal regression. In this new configuration, the DMN-TPN connectivity pattern presented a positive correlation, both before and after Ayahuasca intake. Although there is a trend of increased positive correlation, again no significant difference was found in the DMN-TPN relationship after Ayahuasca intake ($p = 0.22$) (Fig. 3C).

Discussion

Our data demonstrate that Ayahuasca intake leads to a decrease in the activity of core DMN structures.

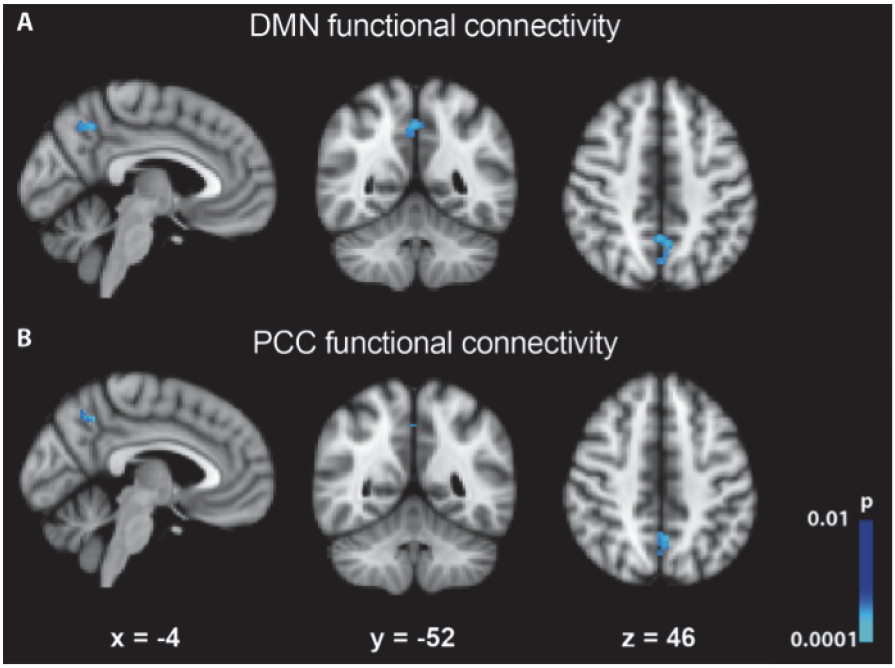

Fig 2. Changes in functional connectivity of DMN. (A) Connectivity within the PCC/Precuneus decreased after Ayahuasca ingestion. (B) Considering only the PCC seed, we can observe that this seed drives the contribution for the decrease in DMN connectivity. Images were thresholded using a cluster corrected pcluster < 0.01 (using a voxel collection threshold of $p < 0.001$).

doi:10.1371/journal.pone.0118143.g002

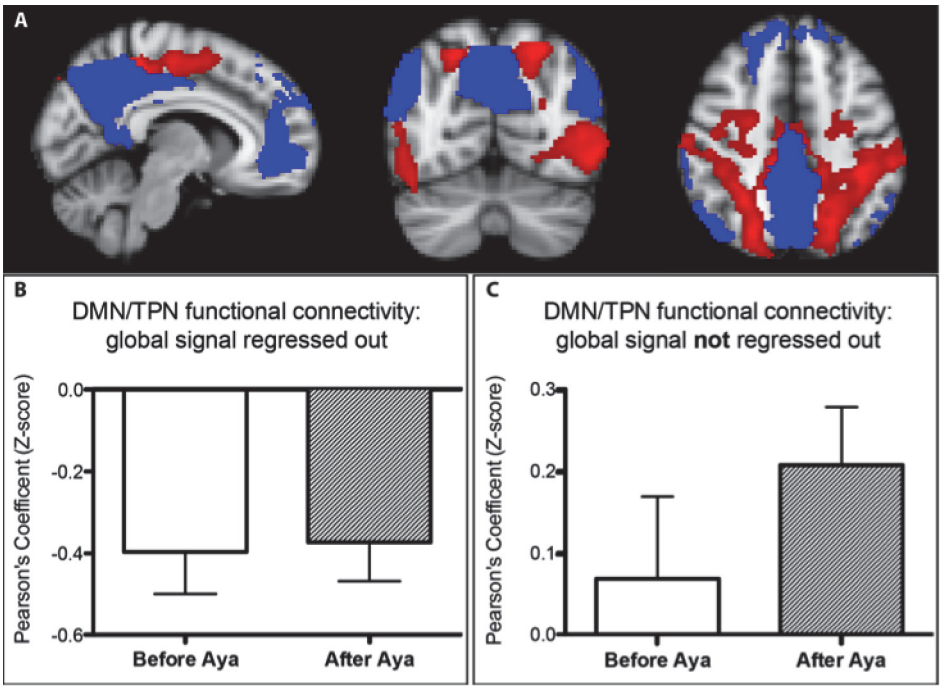

Fig 3. Task Positive and Default Mode Networks. (A) TPN (red) and DMN (blue) masks are shown. (B) TPN and DMN were anti-correlated when the global signal was regressed out, no significant alterations are observed following Ayahuasca intake. (C) Without regression against global signal, TPN and DMN were positively correlated and no significant changes were observed after Ayahuasca ingestion.

doi:10.1371/journal.pone.0118143.g003

There are at least two not mutually excluding hypotheses to explain these results. The first one relates to the very definition of the DMN as a network that disengages when individuals are performing a cognitive task. It is possible that the decrease in DMN activity after the intake of psychedelics comes from the level of concentration and mind effort demanded during the Ayahuasca experience [1]. For instance, religious and shamanic traditions refer to the Ayahuasca experience as "labor". Therefore, it is likely that resting subjects under the influence of psychedelics deal with the experience in a very individual manner, effectively performing internally-generated "labor" equivalent to a task, especially if we consider that the subjects investigated were all experienced users [16].

The second hypothesis stems from the consistent evidence that DMN activity is also reduced during meditative states [13,17,44]. In fact, psychedelics and meditation share many psychological features. For instance, both conditions increase introspection, self-perception, and affect mind-wandering [1,11,45]. Meditation has been described as "the *practice* of resting your attention on whatever is going through your mind, without the attempt of interfering or attaching to the stream of thoughts and emotions" [46]. Decreased DMN activity during meditation has been linked to a decrease in mind-wandering [17]. This should not be the case with psychedelics, as experienced users show potentiated mind-wandering [16]. On the other hand, the awareness of mind-wandering is altered in both states. A recent study suggests that DMN activity increases during periods of mind-wandering, but decreases with the awareness that the mind has wandered [47], and this could be just the case of the Ayahuasca experience. It is as if these experiences lead to a change of standpoint, shifting one's perspective from actor to an attentive spectator.

Although there are similarities, each experience has its own particularities. In fact, this is reflected by our own fc-fMRI results, in particular the observed reduction of functional connectivity within the PCC/Precuneus, while the mPFC-PCC functional connectivity was not significantly altered. In its turn, meditation has been consistently associated with stronger coupling between anterior and posterior nodes of the DMN [17,31,48].

Lately, fc-fMRI has also served as potential marker of different altered states of consciousness, particularly due to changes over posterior midline nodes of the DMN, such as the PCC/Precuneus. For instance, transition from wakefulness to the N1 stage of sleep is marked by decreased connectivity of the PCC with the rest of the DMN, while mPFC connectivity exhibits a stepwise decrease as sleep depth increases [15]. An analogous reduction in PCC connectivity is observed during light sedation with midazolam [32].

Although overall similar to the changes observed for psilocybin, the changes induced by Ayahuasca did not find a significant reduced coupling between PCC and mPFC, as observed after psilocybin intake [16]. Again, although Ayahuasca and psilocybin have much in common, the uniqueness of the experience brought by each substance should be remarked. The Ayahuasca experience usually involves much stronger somatic and sedation effects. From the neuropharmacology perspective, psilocybin acts almost exclusively on the serotonergic system, while Ayahuasca is linked to a rich combination of neurochemical mechanisms: DMT is a trace amine with affinities to sigma-1, monoaminergic, and trace amine-associated receptors [5,8,49,50]. Furthermore, Ayahuasca contains inhibitors of mono-amino-oxidases, which prevents the degradation of monoamine neurotransmitters and thus increase their levels.

DMN signal reduction in the ACC showed a tendency of negative correlation after correction for multiple comparison ($p < 0.072$) with individual YMRS scores. This scale is designed to access changes on mood, sleep, irritability, speech, language-thought, among other manic symptoms [51]. The impact of Ayahuasca on the YMRS scores were mainly influenced by mood, affect, speech, and content of thoughts, and did not indicate a more generalized mania-like state (S5 Fig.). This finding suggests that the subjective changes caused by Ayahuasca,

particularly in the content and structure of thoughts, is associated with the modulation of the anterior part of the DMN.

In addition to investigating the changes in the activity and connectivity of the DMN, we also examined its interaction with the TPN. When the global signal was regressed out, the DMN was anti-correlated to the TPN (Fig. 3B), as expected [36]. Such anti-correlation pattern did not change significantly after Ayahuasca intake. This contrasts with evidence that altered states of consciousness produced by propofol [52], psilocybin [35] and meditation [53] involve a substantial impact on the orthogonality of these networks. It is however worth noting that some of these studies included correction for global signal fluctuations, and this has been lately pointed out as a potential problem when inspecting negative correlations from rs-fMRI. To address this problem, we re-inspected our data keeping the global signal. A positive DMN-TPN correlation emerged, but again no significant correlation change was found after Ayahuasca intake (Fig. 3C). The validity of regressing out the global signal to evaluate DMN-TPN orthogonality is still a matter of debate [41–43,54]. In fact, there is a mounting body of evidence suggesting that the intrinsic DMN-TPN anti-correlation may result from artifacts introduced by regression against global signal [41,42], as we have observed.

The present study concludes that the acute effects of Ayahuasca are associated with diminished DMN activation and decreased functional connectivity of the PCC/Precuneus. Altogether, our results support the notion that the altered state of consciousness induced by Ayahuasca, like with psilocybin, meditation and sleep, is linked to the modulation of the activity and the connectivity of the DMN.

Limitations

This study has some caveats and limitations. First, we limited the study to experienced users. This decision was made to avoid vomiting and diarrhea inside the scanner, two effects frequently observed in naïve subjects. Therefore, the current data cannot clarify whether the effects observed were solely due to the chemical properties of Ayahuasca, or whether previous experience also played a significant role. Second, we did not include a control group treated with a placebo, because it is difficult if not impossible to develop a placebo credible for experienced users, i.e. a substance void of psychoactive chemicals but able to match Ayahuasca's strong and particular flavor. However, it is worth nothing that a number of other studies have already demonstrated that the robust psychological, electrophysiological and neuroimaging [8,11] changes induced by Ayahuasca intake are not explained by a typical placebo effect. The fact that the scans took place inside a hospital, away from the traditional setting of Ayahuasca intake for experienced subjects, also weakens the placebo hypothesis. Third, fixed effects analysis was used, which may limit the results to the group of subjects studied. Forth, the acquisition of fMRI signal was not controlled for order effects. Again, the magnitude of the observed changes is very robust, encompassing six hubs of the DMN. Furthermore, the two sessions were both in the same day, only two hours apart. This protocol guarantees that a stable mental condition of the subjects in both sessions and naturally forbids the control for order, especially for a substance with such strong effects. Unraveling the contributions of chemistry, belief and ritual setting for the hallucinogenic effects of Ayahuasca will require further experimentation. Future studies with larger samples and comparing long-term versus occasional Ayahuasca users are also desired and opportune.

Supporting Information

S1 Fig. DMN mask. This mask was obtained from a control group (26 individuals) that performed the same verbal fluency task as the experimental group. Images were thresholded at q

[FDR] < 0.01, cluster size of 50 contiguous voxels.
(TIF)

S2 Fig. DMN maps before and after Ayahuasca intake. Images were thresholded using cluster corrected pcluster < 0.05 (using a voxel collection threshold of $p < 0.001$).
(TIF)

S3 Fig. Mean β-values from ROIs of the DMN. Bar plots of the mean β-values from ROIs of the DMN where BOLD signal was altered after Ayahuasca ingestion. Decreases were found in most of ROI: ACC, MPFC, PCC, PC and bilateral IPL. $*p < 0.01$ correct for multiple comparisons by the number of ROI (nine).
(TIF)

S4 Fig. Functional connectivity maps obtained for the PCC and mPFC seeds independently. Maps are shown at the two moments: before (left side) and after (right side) Ayahuasca ingestion. The resulting statistical maps were thresholded at $p < 0.05$ uncorrected.
(TIF)

S5 Fig. YMRS changes. Only 5 out of the 11 YMRS items showed statistically significant changes after Ayahuasca intake. Bars present score values (mean + SE). $P < 0.05$ uncorrected.
(TIF)

Acknowledgments

We would like to express our gratitude to the Santo Daime Church, especially to Mestre Pelicano and Jacy, who provided the Ayahuasca and gently helped to recruit the subjects. We also thank Edilson Fernandes da Silva for fruitful discussions.

Author Contributions

Conceived and designed the experiments: DBA SR. Performed the experiments: DBA JAC JH. Analyzed the data: FPF KCA DBA. Wrote the paper: FPF LFT SR DBA. Conducted a neuroradiological analysis of the data: ACS.

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APÊNDICE E – Artigo publicado durante o doutorado

European Neuropsychopharmacology (2015) 25, 483–492

www.elsevier.com/locate/euroneuro

Long-term use of psychedelic drugs is associated with differences in brain structure and personality in humans ☆

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Received 14 August 2014; received in revised form 30 December 2014; accepted 10 January 2015

KEYWORDS
Ayahuasca;
Psychedelics;
N,N-dimethyltryptamine;
Cortical thickness;
Personality

Abstract
Psychedelic agents have a long history of use by humans for their capacity to induce profound modifications in perception, emotion and cognitive processes. Despite increasing knowledge of the neural mechanisms involved in the acute effects of these drugs, the impact of sustained psychedelic use on the human brain remains largely unknown. Molecular pharmacology studies have shown that psychedelic 5-hydroxytryptamine $(5HT)_{2A}$ agonists stimulate neurotrophic and

☆Cortical thickness and psychedelic drugs.

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http://dx.doi.org/10.1016/j.euroneuro.2015.01.008
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transcription factors associated with synaptic plasticity. These data suggest that psychedelics could potentially induce structural changes in brain tissue. Here we looked for differences in cortical thickness (CT) in regular users of psychedelics. We obtained magnetic resonance imaging (MRI) images of the brains of 22 regular users of ayahuasca (a preparation whose active principle is the psychedelic $5HT_{2A}$ agonist *N,N*-dimethyltryptamine (DMT)) and 22 controls matched for age, sex, years of education, verbal IQ and fluid IQ. Ayahuasca users showed significant CT differences in midline structures of the brain, with thinning in the posterior cingulate cortex (PCC), a key node of the default mode network. CT values in the PCC were inversely correlated with the intensity and duration of prior use of ayahuasca and with scores on self-transcendence, a personality trait measuring religiousness, transpersonal feelings and spirituality. Although direct causation cannot be established, these data suggest that regular use of psychedelic drugs could potentially lead to structural changes in brain areas supporting attentional processes, self-referential thought, and internal mentation. These changes could underlie the previously reported personality changes in long-term users and highlight the involvement of the PCC in the effects of psychedelics.
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1. Introduction

Psychedelics, have been used since ancient times by geographically distant human groups for their capacity to induce profound modifications in the ordinary state of consciousness and to generate spiritually meaningful experiences (Schultes, 1979). The ancient practice of ritual psychedelic use not only survived well into the twentieth century, but has expanded beyond indigenous use following contact of previously isolated groups with foreigners (Tupper, 2008).

One of the most interesting contemporary adaptations of psychedelic use is the syncretism observed in Brazilian religious groups that consume ayahuasca, an infusion of the plants *Banisteriopsis caapi* and *Psychotria viridis*. These ayahuasca religions have expanded in the last decades, and it is estimated that around 20,000 people in 23 countries currently take ayahuasca regularly within a ritual context. Typically, participants attend ayahuasca-using rituals once every other week for many years (Grob et al., 1996).

Regarding the active principles present in ayahuasca, one of the plants, *P. viridis*, contains *N,N*-dimethyltryptamine (DMT). DMT is structurally related to serotonin and acts as a $5\text{-}HT_{2A}$ receptor agonist (González-Maeso and Sealfon, 2009). DMT elicits intense, short-acting psychedelic effects when administered intravenously (Strassman et al., 1994) but is rapidly degraded by monoamine-oxidase (MAO) when orally ingested. Interestingly, DMT is rendered orally active by the MAO-inhibiting β-carboline alkaloids found in the other plant, *B. caapi*, used in ayahuasca (Riba et al., 2001).

Molecular pharmacology studies have shown that psychedelic $5\text{-}HT_{2A}$ agonists stimulate expression of immediate early genes that encode transcription factors, such as c-fos (Frankel and Cunningham, 2002), egr-1 and egr-2 (González-Maeso et al., 2007). They also increase the expression of the brain-derived neurotrophic factor (Gewirtz et al., 2002). Activation of these transcription factors has been associated with synaptic plasticity (O'Donovan et al., 1999), and cognitive processes such as memory (Jones et al., 2001) and attention (DeSteno and Schmauss, 2008).

Despite increasing research into the acute effects of psychedelics and the growing interest for their potential use as therapeutic agents (Grob et al., 2011), little is known about the impact of sustained psychedelic use on the human brain. Based on the available molecular data mentioned above, we postulated that repeated exposure to psychedelics would correlate with changes in brain structure. To test this hypothesis we investigated brain cortical thickness (CT) in chronic psychedelic drug users who had minimal exposure to other drugs and their matched controls.

2. Experimental procedures

2.1. Ethical approval of the study protocol

The study protocol was approved by the Ethics Committee at Hospital de Sant Pau (Barcelona, Spain). All participants provided written informed consent to participate in the study.

2.2. Participants

A group of 22 Spanish ayahuasca users and 22 controls were selected for the study. Ayahuasca users were Santo Daime church members who regularly participated in the rituals and were contacted directly by the researchers. Inclusion criteria were: (a) use of ayahuasca at least 50 times in the previous two years; (b) no personal history of psychiatric or neurological disorders; (c) lifetime use of cannabis on twenty occasions or less; (d) lifetime use of other drugs on ten occasions or less; and (e) no use of ayahuasca or other drugs for two weeks before scan, verified by urine toxicology test. The use of ayahuasca of 50 times in two years is a frequency of use of once every other week, which is typical for the Santo Daime church. To rule out a history of psychiatric and neurological disorders, users and controls were interviewed by a clinical psychologist (JCB). Study participants were specifically questioned if they had suffered from depression, psychotic disorders, drug dependence, loss of consciousness, or seizures at any time in their lives. Additionally, at the time of scanning, the structural MRI images were assessed by a neuroradiologist to rule out any CNS anomalies. The two participant groups were matched for sex, age, years of education, and verbal and fluid intelligence quotient (IQ). Each group comprised six male and 16 female participants. The verbal IQ test used was a Spanish version of the NART (Nelson and O'Connell, 1978), known as TAP—"Test de Acentuación de Palabras" ("Word Accentuation Test") (DelSer et al., 1997). The fluid IQ test

used was a computerized version of the Matrix Reasoning from the Wechsler Adult Intelligence Scale (WAIS)-III (Wechsler, 1981).

Table 1 shows sociodemographic data. Between-group comparisons using independent samples Student's *t*-tests and $\chi 2$ did not show any significant differences for any of the matching variables used. Neither were differences found between groups regarding employment, marital status, or tobacco use. However, the number of individuals using tobacco and alcohol was larger in the control group and they also showed a higher frequency of use. Despite the differences in absolute numbers, the $\chi 2$ tests only showed statistical trends.

Ayahuasca users had taken ayahuasca an average of 123 times (range: 50–352). They had been using ayahuasca for an average of 5.3 years (range: 2–13) and the age of initial use was 35.6 years (range: 5–55). Two of the participants had started taking ayahuasca at ages 5 years and 10 years, respectively. None of the participants in either group was currently using cannabis but some had consumed it in the past. As indicated above, maximum lifetime use of twenty times was established as an inclusion criterion and exposure to other drugs of abuse was limited to a maximum of ten times. Four participants in the ayahuasca sample and three in the control group had taken cocaine on less than ten occasions in their lifetime. Four participants in the control group reported having used psychedelics other than ayahuasca once in their lifetime. The drugs used were LSD (one participant) and *Psilocybe* mushrooms (three participants). Urine samples collected on the experimental day were negative for alcohol, benzodiazepines, amphetamines, cannabis, opiates and cocaine for all participants.

2.3. Acquisition and analyses of images

Structural images of the brain were acquired on a 3-T scanner (Magnetom Trio; Siemens, Munich, Germany) using a T1-weighted MPRAGE sequence with the following parameters: 240 sagittal slices; matrix size, 256×256; voxel resolution, 1 mm^3; TR, 2300 ms; TE = 1 ms. CT was estimated using FreeSurfer v5.0.0 in a Mac-Pro OS X 10.8.2, 2×2.26 GHz, Quad-Core Intel Xeon. Images were resampled into common space using a spherical coordinate system, and spatially smoothed with a Gaussian filter (FWHM = 10 mm).

A general linear model (GLM) was applied to estimate statistical differences at each voxel across the entire cortical surface. CT was set as the dependent variable and the group (ayahuasca users and controls) as the discrete factor. To control for possible global differences, the average CT of entire hemispheres was used as a covariate in the GLM model. Results were mapped onto the inflated white-matter surface of the average reconstruction of the brain. Differences between groups were calculated using two-tailed Student's *t*-tests at a statistical threshold of $p < 0.002$ uncorrected and a spatial threshold of ≥ 20 voxels. A parcellation atlas was used to identify the brain structures showing significant differences (Destrieux et al., 1998; Fischl et al., 2004). Statistical maps were color-coded. Brain structures that exhibited significant cortical thinning were represented in "cold" colors (blue-cyan), whereas regions with significant cortical thickening were represented in "hot" colors (red-yellow).

2.4. Assessment of personality, psychopathology and neuropsychology

Personality was assessed using a Spanish version of the Temperament and Character Inventory-Revised (TCI-R) questionnair. The TCI-R is a 240-item questionnaire based on the psychobiological model of personality developed by Cloninger et al. (1993). The four primary dimensions of temperament are: harm avoidance (HA), novelty seeking, reward dependence, and persistence. The three primary dimensions of character are: self-directedness, cooperativeness and self-transcendence (ST).

Psychopathological assessment was carried out using the Symptom Check-List-90-Revised (SCL-90-R) questionnaire. This is a self-report questionnaire comprising 90 Likert-type items distributed in nine symptomatic dimensions: somatization; obsessive–compulsive; interpersonal

Table 1 Sociodemographic data as means (standard deviation) for age, years of education, WAIS matrices score (fluid IQ) and TAP score (verbal IQ). The statistic and *p* value columns show the results for the between-group comparisons (ayahuasca vs. controls) using Student's *t* tests (age, years of education, WAIS and TAP score) and χ^2 tests (all other variables).

	Ayahuasca users	Controls	t/χ^2	*p* Value
Matching variables				
n (Men/women)	6/16	6/16	0.00	NS
Age (years)	40.9 (12.6)	41.5 (11.8)	−0.15	NS
Years of education	13.0 (3.3)	13.1 (3.1)	−0.14	NS
WAIS matrices score	15.7 (3.5)	15.7 (3.6)	0.00	NS
TAP score	25.9 (3.5)	25.0 (3.7)	0.80	NS
Additional sociodemographic variables				
Employment				
employed/unemployed/student	20/0/2	20/0/2	0.00	NS
Marital status				
not married/married	18/4	16/6	0.52	NS
Tobacco and alcohol use				
n Current smokers	2	4	0.77	NS
n Current alcohol drinkers	15	20	3.49	0.062
Pattern of alcohol use				
>1 Drink/day (wine/beer)	0	1	6.58	0.087
1 Drink/day (wine/beer)	0	4		
<1 Drink/day (wine/beer)	1	4		
<1 Drink/week (wine/beer)	14	11		

NS: Not significant.

sensitivity; depression; anxiety; hostility; phobic anxiety; paranoid ideation; psychoticism. The scale also provides three global psychopathological indices: General Severity Index, Positive Symptoms Distress Index and Positive Symptoms Total. For all scales, higher scores imply worse symptomatology (Derogatis, 1994). A Spanish version of the questionnaire was used.

Three classic neuropsychological tests were administered by computer: (a) two-back test to assess working memory (Kircher, 1958); (b) Wisconsin Card-sorting Test (WCST) to assess executive function (including planning, set shifting and inhibition of impulsive responses (Heaton et al., 2001)); and (c) switching task assessing set-shifting (Zimmermann and Fimm, 1994).

The two-back task involved presentation of a sequence of letters. Participants had to identify if the letter was presented two steps back and make a yes/no decision. The series used comprised 100 items, 30% of which were targets.

In the WCST, a pack of 48 cards was presented sequentially on a computer screen. The participants had to classify them according to shape, color or number depending on the active rule. Responses were given by pressing a button. The rule changed after a fixed number of correct classifications. The following variables were assessed: total number of correct responses; total number of errors; total number of perseverances; reaction time for correct responses.

The switching task comprised 100 trials and was based on that described by Zimmermann and Fimm (1994). In each trial, a letter-digit or digit-letter pair is presented on the screen. From one trial to the next, the target item switches from letter to digit and vice versa in the following pattern: letter-digit-letter-digit… or digit-letter-digit-letter… until completion of the 100 trials. Participants have to indicate by button press where on the screen (left or right) the target item is located. Each trial requires a switch of the attention focus from letter to digit and vice versa. In some trials, the change of target type and change in response hand coincide (easy switch condition), and in other trials they do not (hard non-switch condition). Switching of the response hand (easy switch condition) is associated with shorter reaction times (Zimmermann and Fimm, 1994). The variables assessed were percentages of correct non-switch responses, erroneous non-switch responses, correct switch responses, and erroneous switch responses.

Personality, psychopathology and neuropsychological performance data were tested for normality using the Kolmogorov-Smirnov test. This test showed that personality scores (TCI) were normally distributed, whereas psychopathology (SCL-90-R) and neuropsychology (two-back, WCST and task-switching) were not. Thus, for TCI data, mean and standard deviations (SD) were calculated for each subscale and group, and differences between groups were assessed using the Student's *t*-test for independent samples. For the SCL-90-R questionnaire and the neuropsychological tests, medians and ranges were calculated and differences between groups assessed using the non-parametric Mann-Whitney test. Correlations were calculated using Pearson's correlation coefficient for normally distributed data and Spearman's correlation coefficient for non-normally distributed data. Results were considered statistically significant for $p<0.05$.

3. Results

3.1. Structural MRI and CT

Fig. 1 shows the CT statistical maps for the comparison between ayahuasca users and their matched controls. Table 2 shows all clusters in which CT was found to be significantly different between groups. Thinning was observed in the ayahuasca-using group in six cortical areas: the middle frontal gyrus, the inferior frontal gyrus, the precuneus, the superior frontal gyrus, the posterior cingulate cortex (PCC), and the superior occipital gyrus. On the contrary, thickening was found in the precentral gyrus and in the anterior cingulate cortex (ACC). The table shows cluster information including Talairach coordinates, Brodmann area, number of voxels and maximum *t* values.

3.2. Personality, psychopathology and neuropsychology

Mean scores on the main facets of the TCI-R are shown in Table 3 together with the results of the statistical comparison between groups. A detailed analysis of the various subscales comprising each of the seven dimensions of temperament and character was conducted only if the main scale showed significant differences between groups. Ayahuasca users scored lower than controls in terms of HA [$t(42)=-2.08$, $p=0.044$]. This effect was driven by the lower scores in the "anticipatory worry" subscale [$t(42)=-2.98$, $p=0.005$]. Scores on all other subscales were not significantly different. In addition to HA, ayahuasca users scored significantly higher on ST [$t(42)=5.16$, $p<0.001$]. Further analyses showed that all three subscales comprising this character dimension were significantly higher in the ayahuasca group than in the control group: Self-forgetfulness [$t(42)=3.93$, $p<0.001$], Transpersonal identification [$t(42)=3.52$, $p=0.001$], and Spiritual acceptance [$t(42)=5.94$, $p<0.001$].

Results from the psychopathology assessment are shown in Table 4. No significant differences were found for any of the symptomatic dimensions of the SCL-90-R using the non-parametric Mann-Whitney test.

Neuropsychological results are shown in Table 5. Ayahuasca users scored significantly better than controls in several variables derived from the three administered tests. In the two-back test, only the percentage of false alarms and correct rejections did not differ, comparisons for all other variables

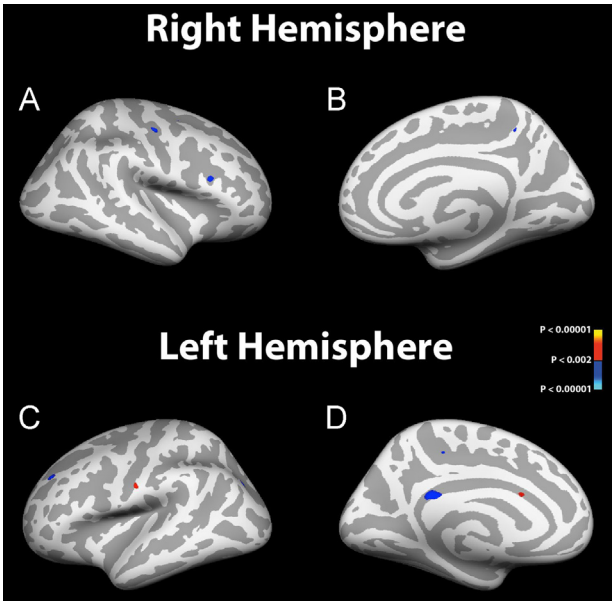

Fig. 1 Statistical maps of cortical thickness (CT) differences between ayahuasca-users and controls displayed onto an inflated cortex. Regions with significantly lower CT in the ayahuasca group are shown in "cold" colors (blue-cyan), and regions with significantly higher CT appear as "hot" colors (red-yellow). Results shown at $p<0.002$ uncorrected and an spatial extension of 20 voxels.

Table 2 Brain areas showing statistically significant differences in cortical thickness between the ayahuasca-using group and the controls at $p<0.002$ uncorrected and a spatial extension of 20 voxels. BA: Brodmann area.

Cortical area	BA	Talairach (*x*, *y*, *z*)	Number of voxels	Maximum *t* value
Ayahuasca < controls				
Middle frontal gyrus	6	(34, −12, 41)	86	−3.538
Inferior frontal gyrus	45	(42, 20, 17)	63	−3.421
Precuneus	7	(9, −42, 46)	23	−3.296
Superior frontal gyrus	9	(−23, 36, 29)	72	−3.538
Posterior cingulate cortex	23	(−4, −38, 23)	166	−3.475
Superior occipital gyrus	19	(−32, −71, 25)	40	−3.314
Ayahuasca > controls				
Precentral gyrus	4	(−57, −9, 25)	53	3.296
Anterior cingulate cortex	24	(−4, 17, 20)	20	3.084

Table 3 Statistical analyses of the six main dimensions of the temperament and character inventory (TCI) questionnaire and of the subscales of the dimensions showing significant differences between groups. Between-group comparisons were carried out using Student's *t* tests. Data are the mean (SD).

TCI	Ayahuasca users	Controls	*t* (*df*=42)	*p*
Novelty seeking	105.2 (10.9)	100.2 (11.8)	1.45	NS
Harm avoidance	86.3 (13.4)	95.3 (15.3)	−2.08	0.044
Reward dependence	107.9 (12.1)	103.1 (13.6)	1.23	NS
Persistence	108.2 (13.6)	104.5 (11.5)	0.99	NS
Self-directedness	157.0 (16.1)	147.3 (22.4)	1.65	NS
Cooperativeness	146.1 (13.1)	141.9 (13.9)	1.03	NS
Self-transcendence	92.8 (14.0)	70.0 (15.3)	5.16	<0.001
Subscales of harm avoidance				
Anticipatory worry	23.3 (4.9)	28.4 (6.2)	−2.98	0.005
Fear of uncertainty	23.0 (4.2)	24.2 (4.7)	−0.92	NS
Shyness with strangers	19.1 (4.0)	21.4 (6.1)	−1.49	NS
Fatigability and asthenia	20.9 (4.6)	21.3 (3.9)	−0.32	NS
Subscales of self-transcendence				
Self-forgetfulness	33.0 (6.0)	26.0 (5.8)	3.93	<0.001
Transpersonal identification	27.0 (5.8)	21.1 (5.4)	3.52	0.001
Spiritual acceptance	32.8 (4.3)	22.9 (6.5)	5.94	<0.001

NS: Not significant.

were significant. In the WCST, ayahuasca users showed a trend to a higher number of correct responses and lower errors. None of the other variables differed between groups. Finally, in the task-switching test, the percentage of correct responses was significantly higher and the percentage of errors was lower in the ayahuasca-using group for non-switch trials. No other significant differences were found.

3.3. Correlation analyses

Correlation analyses were conducted between mean CT values within the significant clusters and lifetime use of ayahuasca. Lifetime use of ayahuasca was inversely correlated to CT in the PCC ($r=-0.444$; $p=0.038$) (Fig. 2a). Years of use of ayahuasca also showed a significant negative correlation with PCC ($r=-0.492$, $p=0.020$) and age of initial use showed a statistical trend ($r=0.390$; $p=0.073$) with earlier use being associated with lower CT (Fig. 2b and c, respectively).

ST scores were correlated with CT in the PCC for each participant group independently (ayahuasca users: $r=-0.479$; $p=0.024$; controls: $r=-0.571$; $p=0.005$). Independent correlations were also found for the transpersonal identification subscale (ayahuasca users: $r=-0.545$; $p=0.009$; controls $r=-0.606$; $p=0.003$) (Fig. 3b). No other significant correlations were found.

4. Discussion

We wished to investigate the impact of regular use of a psychedelic drug on brain structure, personality, psychopathology and neuropsychological function in humans. Our results showed differences in CT between users and controls. These differences were most prominent in medial parts of the brain, specifically an increase in CT in the anterior cingulate cortex and a decrease in CT in the posterior cingulate cortex. Personality assessment showed that the two samples also differed with respect to scores on ST, a character dimension of the TCI-R that measures certain aspects of spirituality (Cloninger et al., 1993) and is closely related to openness (McCrae, 2009).

Table 4 Median and ranges for scores on each of the subscales and indices of the SCL-90-R. Between-groups comparisons were carried out using non-parametric Mann-Whitney tests.

SCL-90-R	Ayahuasca users	Controls	z	p
Somatization	0.50 (0.00-1.17)	0.34 (0.00-1.83)	−0.92	NS
Obsessive-compulsive	0.35 (0.00-2.90)	0.75 (0.00-2.00)	−1.12	NS
Interpersonal sensitivity	0.22 (0.00-2.44)	0.33 (0.00-1.78)	−0.20	NS
Depression	0.15 (0.00-2.23)	0.35 (0.00-1.77)	−1.49	NS
Anxiety	0.25 (0.00-1.60)	0.25 (0.00-1.50)	−0.18	NS
Hostility	0.17 (0.00-2.33)	0.09 (0.00-1.00)	−0.65	NS
Phobic anxiety	0.14 (0.00-1.29)	0.00 (0.00-1.71)	−1.23	NS
Paranoid ideation	0.33 (0.00-1.83)	0.25 (0.00-1.00)	−0.85	NS
Psychoticism	0.20 (0.00-1.30)	0.20 (0.00-0.80)	−0.35	NS
General severity index	0.33 (0.00-1.87)	0.35 (0.03-1.41)	−0.35	NS
Positive symptoms distress index	22.00 (00-70.00)	23.00 (3.00-60.00)	−0.20	NS
Positive symptoms total	1.30 (0.00-2.40)	1.33 (1.00-2.12)	−0.72	NS

NS: Not significant.

Table 5 Medians and ranges for scores on the administered neuropsychological tests. Between-groups comparisons were carried out using non-parametric Mann-Whitney tests. Reaction times (RT) are given in milliseconds.

	Ayahuasca users	Controls	z	p
Two-back				
Hits	77.28 (36.36-93.94)	57.58 (12.12-81.82)	−2.58	0.010
False alarms	4.48 (0.00-56.72)	6.72 (0.00-61.19)	−0.94	NS
Misses	18.18 (3.03-63.64)	37.88 (15.15-87.88)	−2.50	0.013
Correct rejections	95.52 (40.30-100.00)	93.28 (37.31-98.51)	−1.05	NS
RT hits	564 (332-896)	644 (461-961)	−2.09	0.037
A-prime	0.92 (0.67-0.98)	0.86 (0.70-0.95)	−2.23	0.026
D-prime	2.25 (0.53-3.43)	1.63 (0.62-3.08)	−2.36	0.018
WCST				
Total correct	35.50 (17.00-45.00)	32.00 (9.00-42.00)	−1.92	0.055
Total errors	3.50 (0.00-25.00)	6.00 (0.00-37.00)	−1.74	0.081
Total perseverances	2.00 (0.00-17.00)	1.00 (0.00-17.00)	−0.09	NS
RT correct	4058 (2660-8489)	4401 (2252-12,645)	−0.75	NS
Task-switching				
% Correct non-switch	99.07 (86.96-100.00)	96.19 (60.00-100.00)	−2.00	0.046
% Error non-switch	0.93 (0.00-13.04)	3.81 (0.00-40.00)	−2.00	0.046
% Correct switch	100.00 (90.48-100.00)	98.13 (74.36-100.00)	−0.88	NS
% Error switch	0.00 (0.00-9.52)	1.87 (0.00-25.64)	−0.88	NS
RT Correct non-switch	1604 (1036-3193)	1659 (1023-3546)	−0.94	NS
RT Correct switch	1432 (980-4259)	1632 (765-2543)	−1.50	NS

WCST, Wisconsin Card-sorting test; NS: not significant.

The association between changes in brain structure and psychedelic use is supported by the correlations found between lifetime use of ayahuasca and CT in the PCC. Greater exposure to ayahuasca was associated with a higher degree of thinning of the PCC. Despite these structural differences, we did not observe increased psychopathology or worse neuropsychological performance in the ayahuasca-using group. These results are in accordance with studies of long-term users that have found little impact in terms of neuropsychological toxicity (Grob et al., 1996). Indeed, those studies point to a decrease in prior maladaptive behaviors such as drug abuse (Fábregas et al., 2010) and to a change in life attitudes and views as characterized by increased spirituality (Bouso et al., 2012).

The medial prefrontal cortex/ACC and PCC have been associated with the acute effects of ayahuasca and other psychedelic agents. Using single-photon emission tomography, regional cerebral blood flow after acute administration of ayahuasca was found to be increased in the medial aspect of the frontal cortex, including the ACC (Riba et al., 2006). These effects were in accordance with data from studies on psilocybin use employing positron emission tomography (Vollenweider et al., 1997). Analyses of electroencephalography sources showed changes in current density in the ACC, but even more so in the PCC (Riba et al., 2004). These electrophysiological findings have been replicated in a magnetoencephalography study of psilocybin use (Muthukumaraswamy et al., 2013). Interestingly, a recent functional magnetic resonance imaging

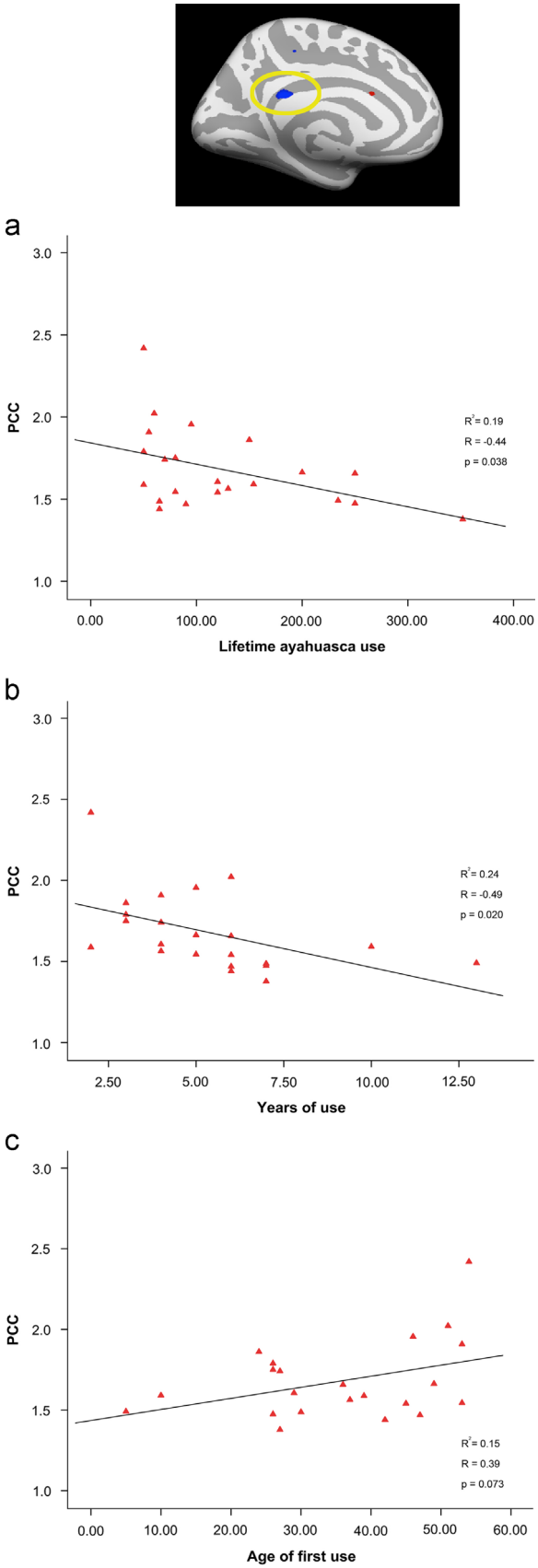

Fig. 2 Scatter plots showing the correlations between individual CT values in the posterior cingulate cortex (PCC) and ayahuasca use: (a) lifetime use; (b) years of use; and (c) age of first use. Talairach coordinates of the cluster center: $x=-4$, $y=-38$ and $z=23$.

study of psilocybin effects found changes in the blood oxygen level-dependent response in the anterior and posterior cingulate cortices and in the functional coupling of these two regions (Carhart-Harris et al., 2012).

From a mechanistic perspective, the structural differences observed in the present study could reflect a direct drug-induced modulatory action or an adaptive response. Supporting the direct-action hypothesis is the fact that activation of 5-HT_{2A} receptors stimulates the expression of immediate early genes (e.g., c-fos) in the medial prefrontal and anterior cingulate cortices (Frankel and Cunningham, 2002). It also increases the expression of brain-derived neurotrophic factor (Gewirtz et al., 2002), which modulates the efficacy and plasticity of synapses (Soulé et al., 2006). Research by González-Maeso and coworkers has shown that psychedelic

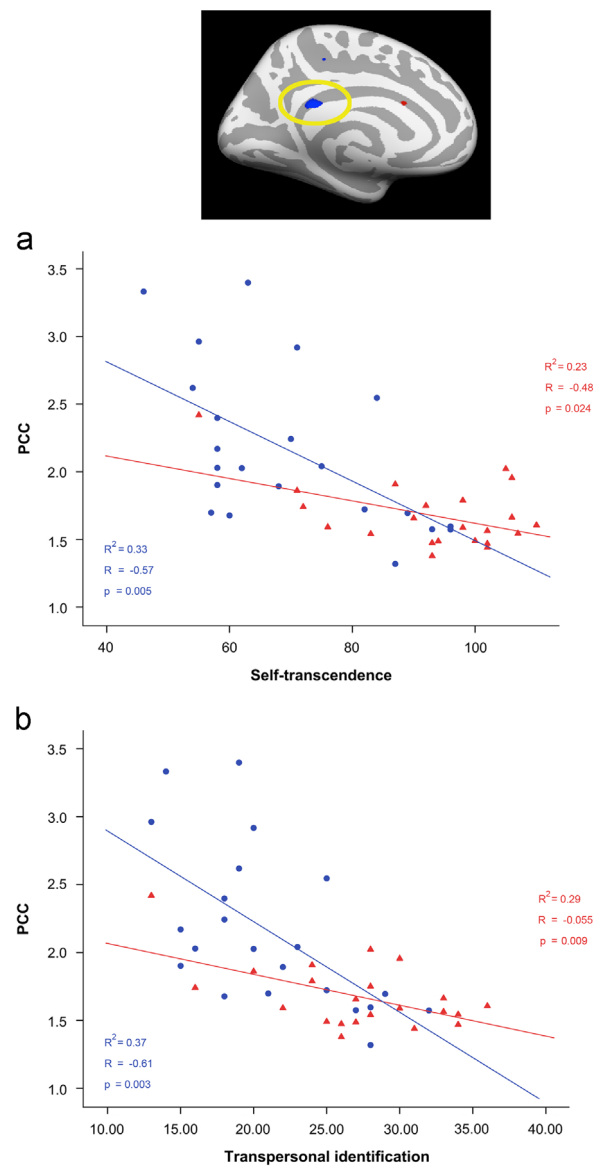

Fig. 3 Scatter plots showing the correlations between cortical thickness in the PCC and personality scores (TCI): (a) with self-transcendence; (b) with the transpersonal identification subscale. Blue dots indicate values for controls and red triangles values for ayahuasca users. Talairach coordinates of the cluster: $x=-4$, $y=-38$ and $z=23$.

5-HT_{2A} agonists induce expression of the transcription factors egr-1 and egr-2 (Moreno et al., 2013). These transcription factors play a prominent part in synaptic plasticity (O'Donovan et al., 1999), and their upregulation and downregulation modulates short- and long-term memory (Jones et al., 2001; Poirier et al., 2008) and attention (DeSteno and Schmauss, 2008). Interestingly, Nichols and coworkers reported that chronic administration of lysergic acid diethylamide leads to altered expression of the genes of dopaminergic and serotonergic receptors long after cessation of drug use (Marona-Lewicka et al., 2011). Thus, it is plausible that the direct pharmacological action of DMT accounts for the observed structural differences after repeated exposure to ayahuasca.

The greater CT observed in anterior brain regions involved with attention and executive control (Raichle, 2011) could explain intriguing findings from experienced users of psychedelic agents. In a study involving 127 drug-free, long-term users of ayahuasca and 115 controls, long-term users scored significantly better on several neuropsychological tests, including the Stroop Test, the WCST and the letter-number sequencing task of WAIS-III (Bouso et al., 2012), indicating that ayahuasca use is not associated with impairment of executive function, and even suggests cognitive enhancement. Another study assessed the impact of prior drug experience on performance during the peak effects of drugs. Authors administered a test battery to two subgroups of users during the acute effects of an ayahuasca dose. They found impaired neuropsychological performance in the "occasional user" subgroup, but not in the "experienced" subgroup (who had taken ayahuasca an average of 180 times) (Bouso et al., 2013). They also reported a correlation between performance in the Tower of London task and lifetime use of ayahuasca. The authors concluded that greater exposure to ayahuasca was associated with less (rather than greater) incapacitation after intake. The present study identified lower CT in the PCC and increases in the ACC, structures involved in the default mode network (DMN) and attention/cognitive control, respectively. These two networks show anti-correlated activity (Fox et al., 2005); crucially, this feature is presumed to be lost under the effects of psychedelics (Carhart-Harris et al., 2012). In the long-term, psychedelic users show opposing structural differences. The observed structural differences at these levels could explain the preservation of neuropsychological function in ayahuasca users.

With regard to personality, we found that ayahuasca users scored higher on ST. This finding is consistent with results in Brazilian users of ayahuasca (Bouso et al., 2012) and work involving acute administration of psychedelic in laboratory settings (Griffiths et al., 2011). Interestingly, greater scores on ST were associated with increased thinning of the PCC. Thus, differences in this character dimension may have a neural basis and be the result of repeated intake of drugs. ST accounts for the tendency towards religiousness and spirituality. ST is believed to be a relatively stable facet, but chronic use of psychedelics was reported to induce personality changes as early as the 1960s and 1970s (Pahnke, 1969; Savage et al., 1966). Researchers described an increased frequency of unusual beliefs and sensations in regular users. It was postulated that psychedelics could cause a profound psychological impression that could lead to changes in attitudes and interests, from less materialistic values to greater open-mindedness and even to mystic-like feelings. These changes were considered positive by some authors (Pahnke, 1969), but others viewed them with concern at a time when use and abuse of these drugs by young adults was more widespread (Blacker et al., 1968). Interestingly, recent research by MacLean et al. (2011) has shown that a single dose of psilocybin can lead to increases in the trait of openness, an aspect of personality that is closely related to ST.

Our observation of a relationship between spirituality and the PCC is of particular note. This brain region is the focus of increasing attention because of its prominent role within the DMN. The network encompassing the ventral precuneus, retrosplenial and posterior cingulate cortices shows high blood flow and energy use under resting conditions (Raichle et al., 2001), and demonstrates extensive structural and functional connections to the other regions comprising the DMN (Horn et al., 2013). This network has been associated with internal mentation and the intimate sense of "self" (Cavanna and Trimble, 2006). The PCC is active during spontaneous stimulus-independent mind-wandering and in processes in which attention is directed internally, such as retrieving episodic memories, imagining and planning (Spreng, 2012). Carhart-Harris et al. (2012) observed deactivation of the PCC after psilocybin administration. They proposed that deactivation of the PCC and the DMN underlies the psychedelic experience, a state that is characterized typically by increased attention to the inner world, i.e., endogenous thoughts and feelings (Riba et al., 2001). The PCC has been proposed to work as a key "hub" regulating information flow around the brain, and its connectivity shows abnormalities in diseases such as schizophrenia (Calhoun et al., 2011), in which the boundaries between internal and external processes become blurred. Interestingly, Carhart-Harris and coworkers reported decreases in functional connectivity between the medial prefrontal cortex and the PCC after psilocybin administration. The results of the present study support involvement of these two areas in the effects of psychedelics.

To conclude, we found that regular use of a psychedelic agent was associated with structural differences in the medial aspects of the frontal and parietal cortices. These differences were associated with prior drug exposure and with greater ST, a personality trait reflecting religiousness and spirituality. Given the cross-sectional nature of the present study, causation cannot be established. However, our data suggest that regular use of psychedelic drugs could potentially lead to changes in brain tissue. Neural changes in brain areas associated with attention, internal thought processes and the sense of self could underlie previously described personality changes following long-term psychedelic use.

Funding

This work was funded by grant 2006/074 from the "Plan Nacional Sobre Drogas" (PNSD) of the Spanish Government. The PNSD had no further role in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

Contributors

JCB conducted the experimental sessions.
FP-F and DBA analyzed the MR data.
ARF designed the neuropsychological test battery.

ARF, SR, RS, JAC and JECH contributed to data interpretation.
JR designed the study, wrote the protocol and drafted the manuscript.
All authors contributed to and have approved the final manuscript.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

The authors thank Saül Martinez-Horta for his help in figure preparation, Amanda Feilding for her critical reading of the paper and Arshad Makhdum from the Beckley Foundation for editing the manuscript.

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